STUDY REPORT

# Antiviral Efficacy Against Virus Infections in Human-Derived Tracheal/Bronchial Epithelial Cells

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| USU Study Number: | 10-20-21-3D XLEAR |
| Report date: | 1 December 2021 |
| Test compounds: | Sorbitol, Erythritol, Xylitol, GSE, Chlorpheniramine Maleate |
| Viruses: | Influenza A/CA/07/09 (H1N1)<br>Respiratory Syncytial Virus (RSV) strain A2<br>SARS-CoV-2 strain USA/PHC658/2021 (B.1.617.2; delta) |

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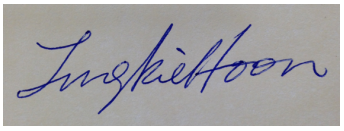

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## Introduction

The antiviral activity of 5 compounds provided by Xlear were evaluated against Influenza A/CA/07/09 (H1N1), Respiratory Syncytial Virus (RSV) strain A2, and SARS-CoV-2 strain USA/PHC658/2021 (B.1.617.2; delta) in a highly differentiated, three-dimensional (3-D), *in vitro* model of normal, human-derived tracheal/bronchial epithelial (TBE) cells. The compounds were tested at the concentrations indicated in Tables 1-3 in duplicate inserts of the 3D tissue models of the human airway (MatTek Life Sciences). Antiviral activity was measured by virus yield reduction assays on day 3 (H1N1), day 5 (RSV), or day 6 (SARS-C-V2) after infection.

## Materials and Methods

*Compounds:* The compounds received as solids were dissolved in the MatTek culture medium (AIR-100-MM) and further diluted to the test dilutions. Sorbitol (45%) and GSE (43%) were received in solution and were further diluted to the test dilutions in the culture medium. Ribavirin (ICN Pharmaceuticals, Inc. Costa Mesa, CA) or Remdesivir (MedChemExpress, cat# HY-104077) were tested as the positive control.

*Cell Culture:* The EpiAirway™ Model consists of normal, human-derived tracheal/bronchial epithelial (TBE) cells which have been cultured to form a multi layered, highly differentiated model which closely resembles the epithelial tissue of the respiratory tract. The cell cultures were made to order by MatTek Life Sciences (https://www.mattek.com) (Ashland, MA) and arrived in kits with either 12- or 24-well inserts each. The TBE cells were grown on 6mm mesh disks in transwell inserts. During transportation the tissues were stabilized on a sheet of agarose, which was removed upon receipt. One insert was estimated to consist of approximately 1.2 x $10^6$ cells. Kits of cell inserts (EpiAirway™ AIR-100, AIR-112) originated from a single, healthy, non-smoker donor #9831. Upon arrival, the cell transwell inserts were immediately transferred to individual wells of a 6-well plate according to manufacturer's instructions, and 1 mL of MatTek's proprietary culture medium (AIR-100-MM) was added to the basolateral side, whereas the apical side was exposed to a humidified 5% $CO_2$ environment. The TBE cells were cultured at 37°C for a minimum of one day before the start of the experiment. After the equilibration period, the mucin layer, secreted from the apical side of the cells, was removed by washing with 400 µL pre-warmed 30 mM HEPES buffered saline solution 3X. Culture medium was replenished to the basal side following the wash steps. The tissues were then allowed to rest in a 37°C and 5% $CO_2$ environment for a minimum of 1 hour prior to the assay.

*Viruses:* The virus stocks were diluted in AIR-100-MM and infected at MOI 0.01 (H1N1), MOI 0.01 (RSV) and MOI 0.02 (SARS-CoV-2) $CCID_{50}$ per cell, respectively.

*Experimental design:* Each compound treatment (140 µL) is applied to the apical side, and culture medium only is applied to the basal side (1 mL), for a 2 h incubation. Virus is then added (140 µL) to the apical side for a 2 h infection period. As a virus control, some of the cells were treated with placebo (cell culture medium only). Following the infection, the apical medium was removed, wells are washed once with media, and fresh test compound is added to the apical side. The basal side was replaced with fresh medium. The cells were

maintained at the air-liquid interface. On days 3 (H1N1), 5 (RSV), or 6 (SARS-CoV-2) post-infection, the medium was removed and discarded from the basal side. Virus released into the apical compartment of the TBE cells was harvested by the addition of 400 µL of culture medium that was pre-warmed at 37°C. The contents were incubated for 30 min, mixed well, collected, thoroughly vortexed and plated on MDCK (H1N1), MA-104 cells (RSV), or Vero E6 cells (SARS-CoV-2) for VYR titration. Triplicate wells were used for virus controls.

*Determination of virus titers from each treated cell culture:* MDCK (H1N1), MA-104 cells (RSV), or Vero E6 cells (SARS-CoV-2) cells were seeded in 96-well plates and grown overnight (37°C) to confluence. Samples containing virus were diluted in 10-fold increments in infection medium and 200 µL of each dilution transferred into respective wells of a 96-well microtiter plate. Four microwells were used for each dilution to determine 50% viral endpoints. After 3-7 days of incubation, each well was scored positive for virus if any cytopathic effect (CPE) was observed as compared with the uninfected control. The virus dose that was able to infect 50% of the cell cultures ($CCID_{50}$ per 0.2 mL) was calculated by the Reed-Muench method (1948). The VYR data and log reduction values (LRV) are summarized in Tables 1-3.

## References

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. The American Journal of Hygiene 27, 493–497.

**Table 1. Antiviral efficacy against Influenza A/CA/07/09 (H1N1)**

| Test Compounds | Concentration (%) | [a]$Log_{10}$ $CCID_{50}$ virus per 0.2 mL | [b]LRV |
|---|---|---|---|
| Sorbitol | 5<br>5 | 6.30<br>5.30 | 1.47 |
| Erythritol | 5<br>5 | 3.50<br>4.67 | 3.17 |
| Xylitol | 5<br>5 | 6.00<br>6.00 | 1.27 |
| GSE | 0.2<br>0.2 | 1.50<br>1.50 | 5.77[d] |
| Chlorpheniramine Maleate | 1<br>1 | 1.50<br>1.00 | 6.02[d] |
| Ribavirin | 100 μg/ml<br>10<br>1 | 0.67<br>5.50<br>7.30 | [c]$EC_{90}$<br>3.1<br>SI >32 |
| Virus Control Influenza A/CA/07/09 (H1N1) | MOI 0.01 | 7.30<br>7.00<br>7.50 | Avg.<br>7.27 |

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.
[a]Titer results from the virus yield reduction assay.
[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control
[c]$EC_{90}$ = 90% effective concentration (reduce virus yield by 1 $log_{10}$) as determined by regression analysis.
[d]Some cell cytotoxicity was observed and may have contributed to the antiviral effects.

**Table 2. Antiviral efficacy against Respiratory Syncytial Virus (RSV) strain A2**

| Test Compounds | Concentration (%) | [a]$Log_{10}$ $CCID_{50}$ virus per 0.2 mL | [b]LRV |
|---|---|---|---|
| Sorbitol | 5<br>5 | 2.00<br>2.00 | 2.49 |
| Erythritol | 5<br>5 | 3.00<br>2.30 | 1.84 |
| Xylitol | 5<br>5 | 2.00<br>1.67 | 2.65 |
| GSE | 0.2<br>0.2 | 2.00<br>2.30 | 2.34[d] |
| Chlorpheniramine Maleate | 1<br>1 | 1.50<br>1.50 | 2.99[d] |
| Ribavirin | 100 μg/ml<br>10<br>1 | 1.30<br>3.30<br>4.67 | [c]$EC_{90}$<br>4.6<br>SI >22 |
| Virus Control<br>RSV A2<br>ATCC VR-1540 | MOI 0.01 | 4.50<br>4.67<br>4.30 | Avg.<br>4.49 |

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.
[a]Titer results from the virus yield reduction assay.
[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control
[c]$EC_{90}$ = 90% effective concentration (reduce virus yield by 1 $log_{10}$) as determined by regression analysis.
[d]Some cell cytotoxicity was observed and may have contributed to the antiviral effects.

**Table 3. Antiviral efficacy against SARS-CoV-2 strain USA/PHC658/2021 (B.1.617.2; delta).**

| Test Compounds | Concentration (%) | [a]$Log_{10}$ $CCID_{50}$ virus per 0.2 mL | [b]LRV |
|---|---|---|---|
| Sorbitol | 5<br>5 | 1.50<br>1.67 | 3.5 |
| Erythritol | 5<br>5 | 1.67<br>2.00 | 3.29 |
| Xylitol | 5<br>5 | 1.50<br>1.00 | 3.84 |
| GSE | 0.2<br>0.2 | 1.50<br>1.50 | 3.59[d] |
| Chlorpheniramine Maleate | 1<br>1 | 2.30<br>2.50 | 2.69[d] |
| Remdesivir | 5 µM<br>0.5<br>0.05 | 0.67<br>2.50<br>5.00 | [c]$EC_{90}$<br>0.12<br>SI >42 |
| Virus Control SARS-CoV-2 USA /PHC658 /2021 (B.1.617.2; delta) | MOI 0.02 | 5.30<br>5.30<br>4.67 | Avg.<br>5.09 |

Each well was scored positive for virus if any CPE was observed as compared with the uninfected control.
[a]Titer results from the virus yield reduction assay.
[b]LRV (log reduction value) is the average reduction of virus compared to the average virus control
[c]$EC_{90}$ = 90% effective concentration (reduce virus yield by 1 $log_{10}$) as determined by regression analysis.
[d]Some cell cytotoxicity was observed and may have contributed to the antiviral effects.