# A High-Throughput Human Tissue Model for Respiratory Viruses

Miranda Nebane, Andrew Reece, Lynn Rasmussen, Melinda Sosa, Pedro Ruiz, Sara M. Cabrera, Yohanka Martinez-Gzegozewska and Paige Vinson.
High-Throughput Screening Center, Scientific Platforms Division, Southern Research, Birmingham AL 35205

## Introduction

Animal testing for drug discovery is expensive and the decision to test a compound in an animal model should be carefully considered. In addition, the FDA Modernization Act has resulted in the allowance of alternatives to animal models for testing the safety and efficacy of drug candidates. Among these alternatives are human tissue models that provide a human-relevant context. Specialized cell types can be produced from primary human cells and used for basic research and drug discovery purposes. One of these is a 3D model for respiratory disease research, consisting of human-derived tracheal/bronchial epithelial cells. Though this translational Human Airway Epithelial (HAE) model is currently being employed by many researchers, this work is primarily done using individual tissue inserts placed in 6-well plates. This low-throughput approach is labor-intensive, time-consuming and expensive (high cost per each compound screened). We have established a high-throughput HAE assay that can be used for compound screening to advance respiratory virus programs. This is significant as it allows a higher number of compounds to be evaluated before being tested in vivo, allowing a more comprehensive comparison of candidates at this later discovery stage. We developed 96-well assays to evaluate compounds for Influenza, Respiratory Syncytial Virus (RSV) and Coronaviruses (including BSL-3 strains such as SARS-CoV-2), and these assays are easily adaptable to other respiratory viruses like Human metapneumovirus (HMPV). The development process involved performing a titration of each virus for $TCID_{50}$ calculations and determining the optimal HAE infection time in a time course experiment, with every step of the process automated to increase speed and accuracy. Following infection of the HAE tissues, the amount of infectious virus in HAE supernatant was assessed in a Cytopathic Effect (CPE) or Virus Titer Reduction (VTR) assay in an appropriate cell line for that particular virus. The optimized assays consistently showed Z' values > 0.7 and were used to test reference compounds relevant to each antiviral assay. The assay development process and representative results for H3N2 A/Udorn/72 and RSV Long strain will be presented to highlight the benefits of miniaturizing *HAE assays* from individual wells into a 96-well format, thereby providing a high-throughput solution for human 3D in vitro respiratory tissue models.

## Advantages of HAE Models

- Human 3D translational model for drug discovery, targeting respiratory viruses
- *In vitro* model that mimics human *in vivo* environment
- Current offerings are predominantly 6-well format (individual tissues in a 6-well plate). These are low-throughput and have a high cost per compound
- 96-well format and automation can characterize more compounds in a cost-effective manner
- Can be adapted for all respiratory viruses…Influenza, RSV, HMPV, Coronavirus, etc.

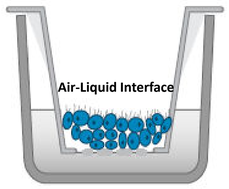
## Considerations

- Each virus strain (e.g. H3N2 A/Udorn/72, H1N1 A/California/07/2009, HCoV-229E) will require a new round of assay development; virus titration for $TCID_{50}$ calculations, coupled with a time course to determine the optimal HAE infection time
- A 96-well liquid handler capable of aspirating and dispensing is important, as the protocol includes wash steps
- Dispense speed should be low enough to avoid disturbing the HAE tissue
- Aspirate height should be low enough to collect most of the liquid in the well, but without touching the HAE tissue
- Not all virus supernatant harvests can be frozen for endpoint assay on a later date. E.g. For RSV (Long strain), the CPE assay needs to be set up using freshly-harvested supernatant from the infected HAE tissues.

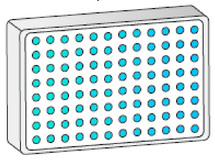
## Discussion

- The Human Airway Epithelial (HAE) model is amenable to HTS
- Miniaturizing HAE assays for performance in 96-well plates increases throughput and is less labor-intensive
- This will allow more compounds to be tested in this human primary cell model, thereby lowering the cost per compound
- Amount of infectious virus in supernatant collected from infected HAE cells can be measured in a plate-based CPE (or other endpoint) assay, which can be in 96-well format or further miniaturized to 384-well format
- After assay optimization, the assay was reproducible for HAE cells infected with H3N2 A/Udorn/72 and H1N1 A/California/07/2009, with Z' values above 0.75.

## General Methodology




Single Well
- Low-throughput
- Tedious, laborious
- High cost per compound



96-Well
- High-throughput
- Automatable
- Cost-effective

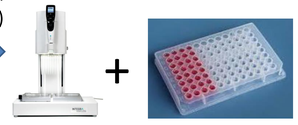
***STEP 1*: TITER THE VIRUS AND DETERMINE OPTIMAL INCUBATION TIME**

Infection of Human Airway Epithelial (HAE) cells and supernatant harvest

Obtain 96-well HAE tissue plate (Mattek; EpiAirway Part # AIR-196-HTS)

Put HAE cells in culture (X days)

37 °C, high humidity



Titrate virus. Use VIAFLO 96 (Integra) for all aspirate and dispense steps

Change media daily

37 °C, high humidity

Collect supernatant daily and freeze at -80 °C

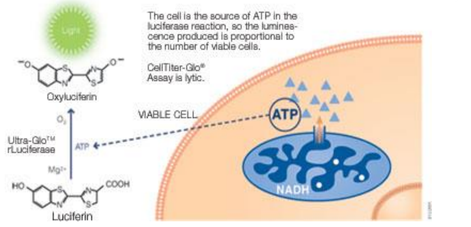
Time course

Assessing amount of infectious virus in HAE supernatant harvest (CPE Assay)

Thaw supernatants from time course, serial-dilute, transfer to TCT 96- or 384-well plate

Add appropriate cell line for CPE assay (X days)

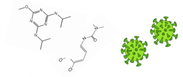
X °C, high humidity




Addition of Promega CellTiter-Glo® allows determination of ATP levels, an indicator of metabolically active cells

***STEP 2*: TEST REFERENCE COMPOUNDS USING OPTIMIZED CONDITIONS**

96-well HAE cells in culture



Add compounds in dose response; Add pre-determined concentration of virus

Incubate 37 °C, high humidity, X days

Harvest Supernatant (and freeze if desired)

CPE Assay

Thaw supernatant and transfer to CPE assay plate. Add pre-determined number of cells

Incubate X °C, high humidity, X days

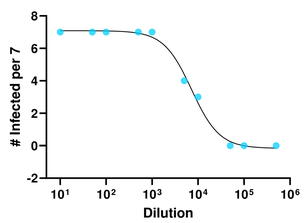
Add CellTiter-Glo, Read plate

## Results from 96-well HAE Cultures

***STEP 1 Results: Titer of* H3N2 A/Udorn/72 in HAE Cells**

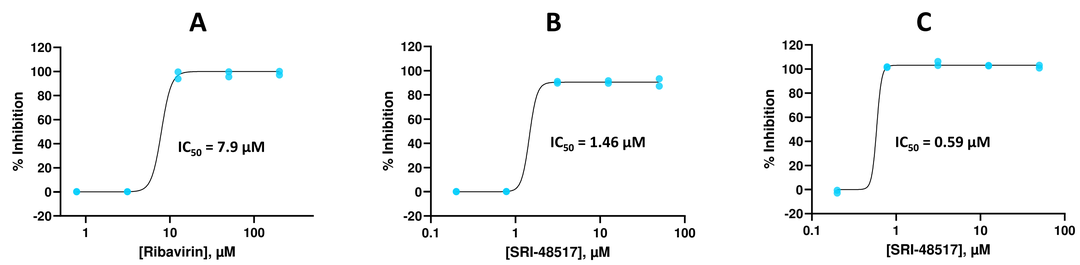
| | NEAT | 10 | 50 | 100 | 500 | 1,000 | 5,000 | 10,000 | 50,000 | 100,000 | 500,000 | CELLS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 513 | 428 | 318 | 251 | 800 | 547 | 943 | 902 | 127472 | 123353 | 130836 | 98224 |
| B | 1602 | 789 | 119 | 500 | 736 | 822 | 124811 | 126389 | 125266 | 126188 | 122494 | 129065 |
| C | 412 | 1633 | 439 | 337 | 764 | 810 | 402 | 3217 | 128155 | 124768 | 122121 | 129928 |
| D | 912 | 1088 | 1209 | 371 | 594 | 755 | 867 | 116773 | 117407 | 116363 | 119503 | 125097 |
| E | 543 | 792 | 783 | 989 | 944 | 1420 | 117984 | 118710 | 114897 | 119880 | 129647 | 132500 |
| F | 714 | 467 | 390 | 880 | 1301 | 1408 | 116447 | 117536 | 116769 | 125270 | 119107 | 128556 |
| G | 1918 | 694 | 580 | 657 | 1000 | 792 | 930 | 1954 | 116412 | 115413 | 123231 | 128484 |
| H | 119886 | 120310 | 123892 | 125550 | 123317 | 121668 | 119990 | 123194 | 118138 | 120049 | 123340 | 129067 |

Heat map of H3N2 A/Udorn/72 Titer Data in 96-well HAE plate. Column 1: Neat virus (MOI = 12). Columns 2 – 11: 1:10 to 1:500,000 virus dilution. Column 12: Cell Control. Row H: 1 µM VX-787 with corresponding virus dilution.




Plot of the number of virus-positive wells out of 7 wells tested versus dilution for $TCID_{50}$ determination.

***STEP 2 Results: Dose Response of Ribavirin and SRI-48517 in* H3N2- and H1N1-Infected HAE Cells**




Human Airway Epithelial (HAE) cells in 96-well format were infected with H3N2 A/Udorn/72 or H1N1 A/California/07/2009. Two days post-infection, harvested supernatant was tested for infectious virus by cytopathic effect (CPE) in MDCK cells. The results shown above are for ribavirin and a proprietary compound, SRI-48517. Experiments are run with compounds in 5-point dose response to determine their potency in HAE cells. n = 2 for compound wells; n = 8 for control wells. **A and B = H3N2 A/Udorn/72; C = H1N1 A/California/07/2009.**

## Acknowledgment

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