EPITHELIX
In vitro respiratory solutions

# *Fingerprint of the most prevalent respiratory viral strains on in vitro primary human nasal epithelium*

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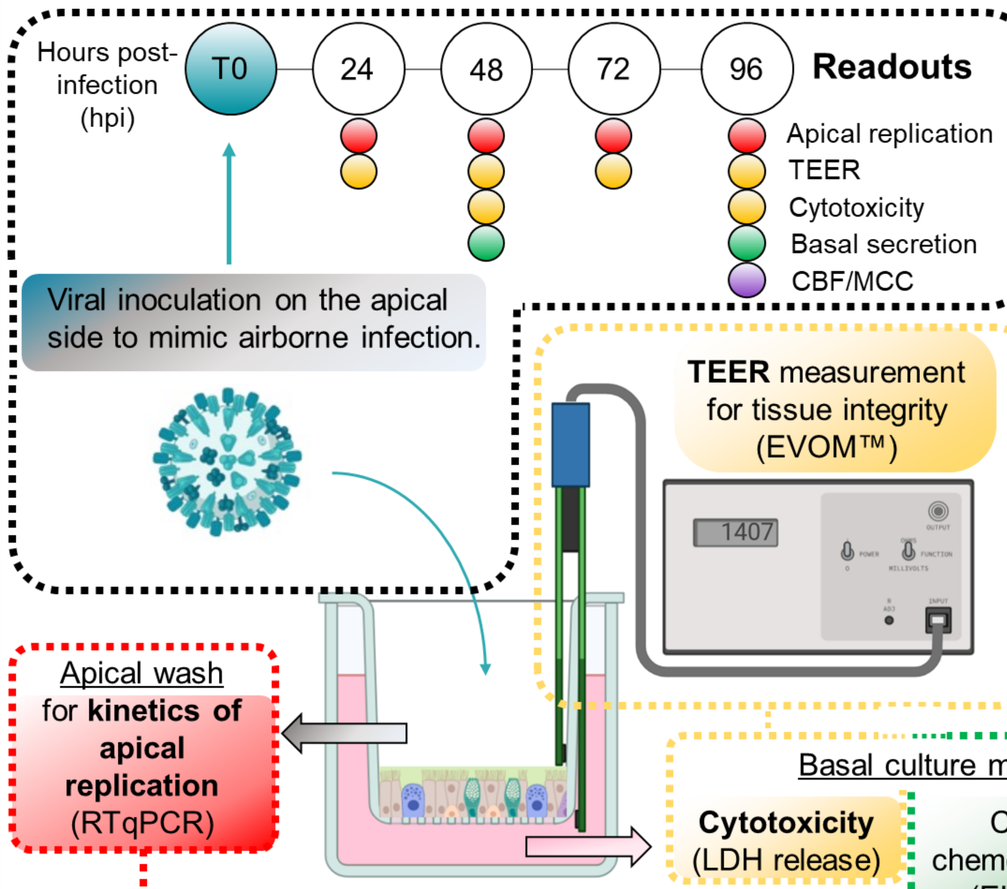
Acute respiratory infections are a leading cause of death worldwide, with an estimated 20% of total death in younger than five years old children. MucilAir™ is a fully differentiated 3D nasal *in vitro* model, reconstituted from human primary cells, that recapitulates key functions of the respiratory epithelium. Here, we assessed the efficacy of antiviral drugs on influenza A (H1N1), respiratory syncytial virus (RSV-A), rhinoviruses (RV-A16), human metapneumovirus (hMPV) and para-influenza virus (PIV3) using human nasal epithelia reconstituted from a pool of 14 donors.

**Tested antivirals** Baloxavir marboxil (Balo), Oseltamivir, Nirmatrelvir, Molnupiravir, Ribavirin, Rupintrivir

**Positive controls** Cytomix (Cyto) for inflammation, Triton for cytotoxicity
**Negative controls** Mock infection (Mock), Non-treated (NT)

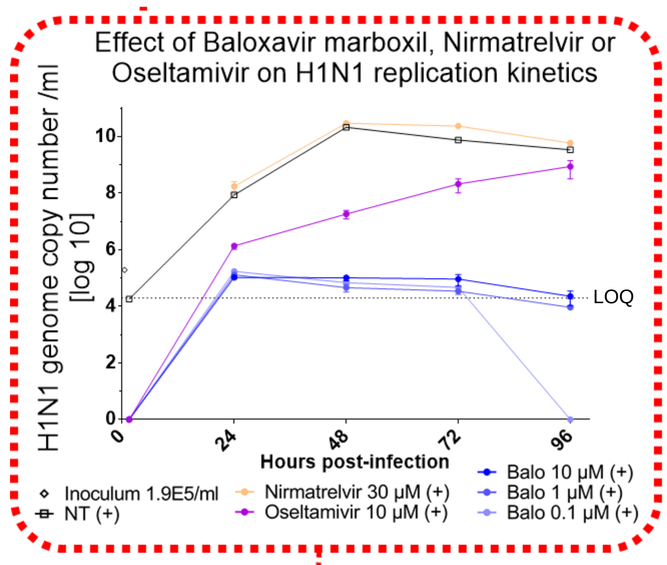
**(+) I**nfected
**(-)** Non-infected

## 1. Establishing viral fingerprint on 3D human nasal epithelium and ranking antiviral drug efficacy : case study with H1N1

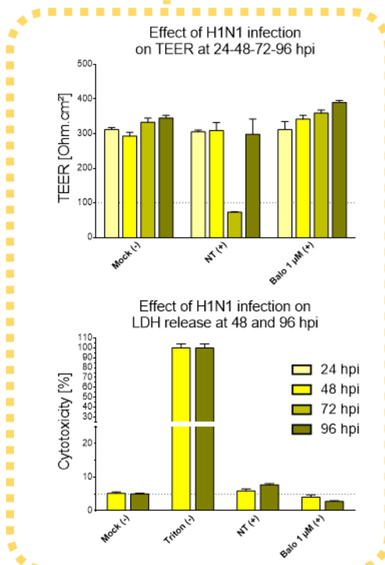


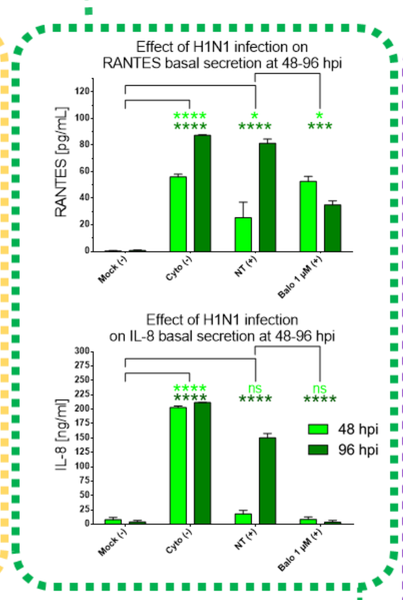

Additional readouts are possible

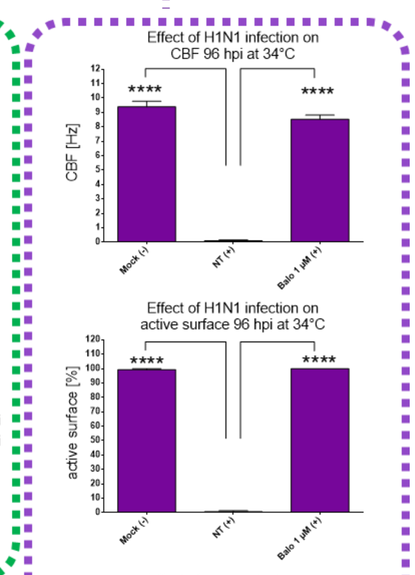
- Tissue lysis for gene expression.
- TCID50 from apical wash (infectivity).
- Tissue fixation for IF/TEM/SEM.
- Cytokines and chemokines from apical wash.
- Permeability / uptake.
- Ion channels activity (Ussing chamber).




H1N1 replicated efficiently in MucilAir™. Baloxavir marboxil (0.1 µM) reduced H1N1 apical replication more efficiently than Oseltamivir (30 µM). Nirmatrelvir (30 µM) had no effect.




**---- H1N1 fingerprint (4 days) ----**

| | | |
|---|---|---|
| **Structural integrity** | TEER | ↓ transient at 72 hpi |
| | Cytotoxicity | 5-10% |
| **Secretion** | RANTES | ↑ at 48 and 96 hpi |
| | IL-8 | ↑ at 48 and 96 hpi |
| **Cilia motion** | CBF | Loss of motion |
| | MCC | Loss of function |

**Conclusion**
H1N1 induces a decrease of TEER associated with a slight cytotoxicity, indicating transient loss of barrier function. Viral infection increases IL-8 and RANTES basal secretion and completely abolishes cilia motion. **Baloxavir marboxil (1 µM)** is the most efficient to reduce H1N1 replication and associated deregulations.

## 2. Fingerprints of RV-A16 or RSV-A infections

**---- RV-A16 fingerprint (4 days) ----**

| | | |
|---|---|---|
| **Structure/ damage** | TEER | No effect |
| | Cytotoxicity | No effect |
| **Secretion** | RANTES | ↑ at 96 hpi |
| | IL-8 | ↑ at 96 hpi |
| **Cilia motion** | CBF | No effect |
| | MCC | No effect (↓ ns) |

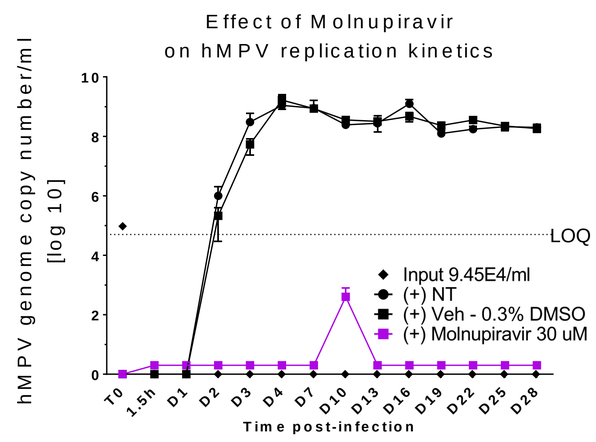
**RV-A16** replication and associated deregulations are efficiently reduced by **5 µM Rupintrivir**.

**---- RSV-A fingerprint (4 days) ----**

| | | |
|---|---|---|
| **Structure/ damage** | TEER | No effect |
| | Cytotoxicity | No effect |
| **Secretion** | RANTES | ↑ at 96 hpi |
| | IL-8 | ↑ at 96 hpi |
| **Cilia motion** | CBF | ↓ at 96 hpi |
| | MCC | ↓ velocity |

**RSV-A** apical replication and associated deregulations are efficiently reduced by **100 µM Ribavirin**.

## 3. Fingerprints of hMPV or PIV3 infection (28 days)




**---- hMPV fingerprint (28 days) ----**

| | | |
|---|---|---|
| **Structure/ damage** | TEER | No effect |
| | Cytotoxicity | No effect |
| **Secretion** | RANTES | No effect |
| | IL-8 | N/D |
| **Cilia motion** | CBF | ↓ transient (D7-16) |
| | MCC | N/D |

**hMPV** replication and associated deregulations are efficiently reduced by **30 µM Molnupiravir**. Ribavirin (100 µM) is slightly less efficient. Nirmatrelvir (30 µM) has no effect.

**---- PIV3 fingerprint (28 days) ----**

| | | |
|---|---|---|
| **Structure/ damage** | TEER | ↓ D4-D28 |
| | Cytotoxicity | No effect |
| **Secretion** | RANTES | No effect |
| | IL-8 | N/D |
| **Cilia motion** | CBF | No effect |
| | MCC | No effect |

**PIV3** replication is efficiently reduced by **30 µM Molnupiravir** or **Nirmatrelvir**. Ribavirin (100 µM) is less efficient.

## CONCLUSION AND SUMMARY

This set of data revealed a strain-specific fingerprint on standardized *in vitro* nasal epithelium (MucilAir™).

This *in vitro* assay allows ranking of antivirals efficacy and toxicity. It can be used as a screening platform for the development of new drugs through systemic or airborne delivery.

| | Most efficient | Less efficient | Inefficient |
|---|---|---|---|
| H1N1 | Baloxavir marboxil 1 µM | Oseltamivir 10 µM | Nirmatrelvir 30 µM |
| RV-A16 | Rupintrivir 5 µM | - | - |
| RSV-A | Ribavirin 100 µM | - | - |
| hMPV | Molnupiravir 30 µM | Ribavirin 100 µM | Nirmatrelvir 30 µM |
| PIV3 | Molnupiravir 30 µM<br>Nirmatrelvir 30 µM | Ribavirin 100 µM | - |