



# Epithelix

*in vitro* Solutions for Respiratory Diseases and Chemical Testing



## The use of *in vitro* Human Airway Epithelia for the development of novel antivirals against human rhinoviruses (HRV)

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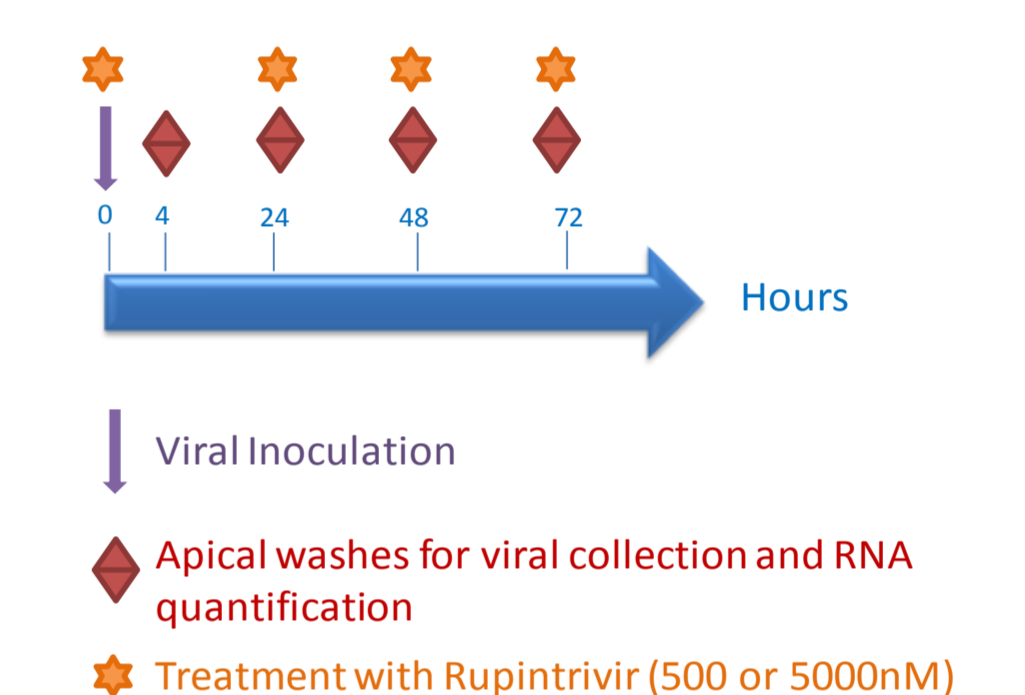
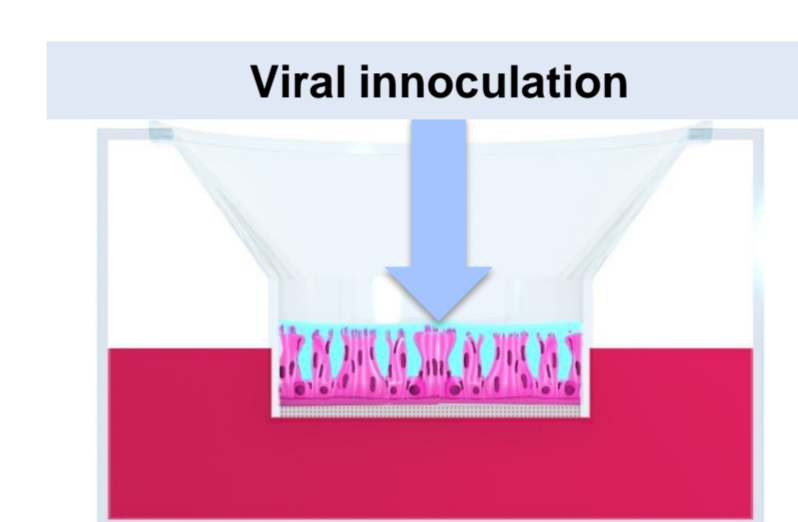
Viruses such as Human Rhinovirus (HRV) are frequently implicated in exacerbations of some respiratory diseases like COPD and Asthma. Development and use of antiviral drugs are one of the priorities for major pharmaceutical companies. However, due to viral tropism, animal models and even human cell lines are not appropriate for propagating and modelling viral infections. It is the case for HRV-C sub-types which cannot be replicated in standard cell lines. To solve this problem, we explored the potential of an *in vitro* tissue culture model of the human airway epithelium (MucilAir™). We report here the efficient infection/ replication of a panel of clinical HRV-C specimens in MucilAir™, including HRV-C2, HRV-C7, HRV-C12, HRV-C15, and HRV-C29. We observed that the virions enter and exit preferentially through the apical surface.

As proof-of-concept for drug screening, the efficacy of Rupintrivir, a commercially available antiviral, was tested in our system: Rupintrivir efficiently inhibited the replication of HRV-A16 and HRV-C15 in a dose and time dependent manner (up to 99% inhibition).

### The advantages of MucilAir™

### Testing Strategy

- It is composed of **primary human respiratory cells**.
- It **mimics** the morphology and functions of the native human airway epithelium.
- It has a **unique shelf-life of 12 months**.
- Epithelia from **different pathologies** are available (asthma, COPD, CF, allergic rhinitis).
- It is **ready and easy to use**.

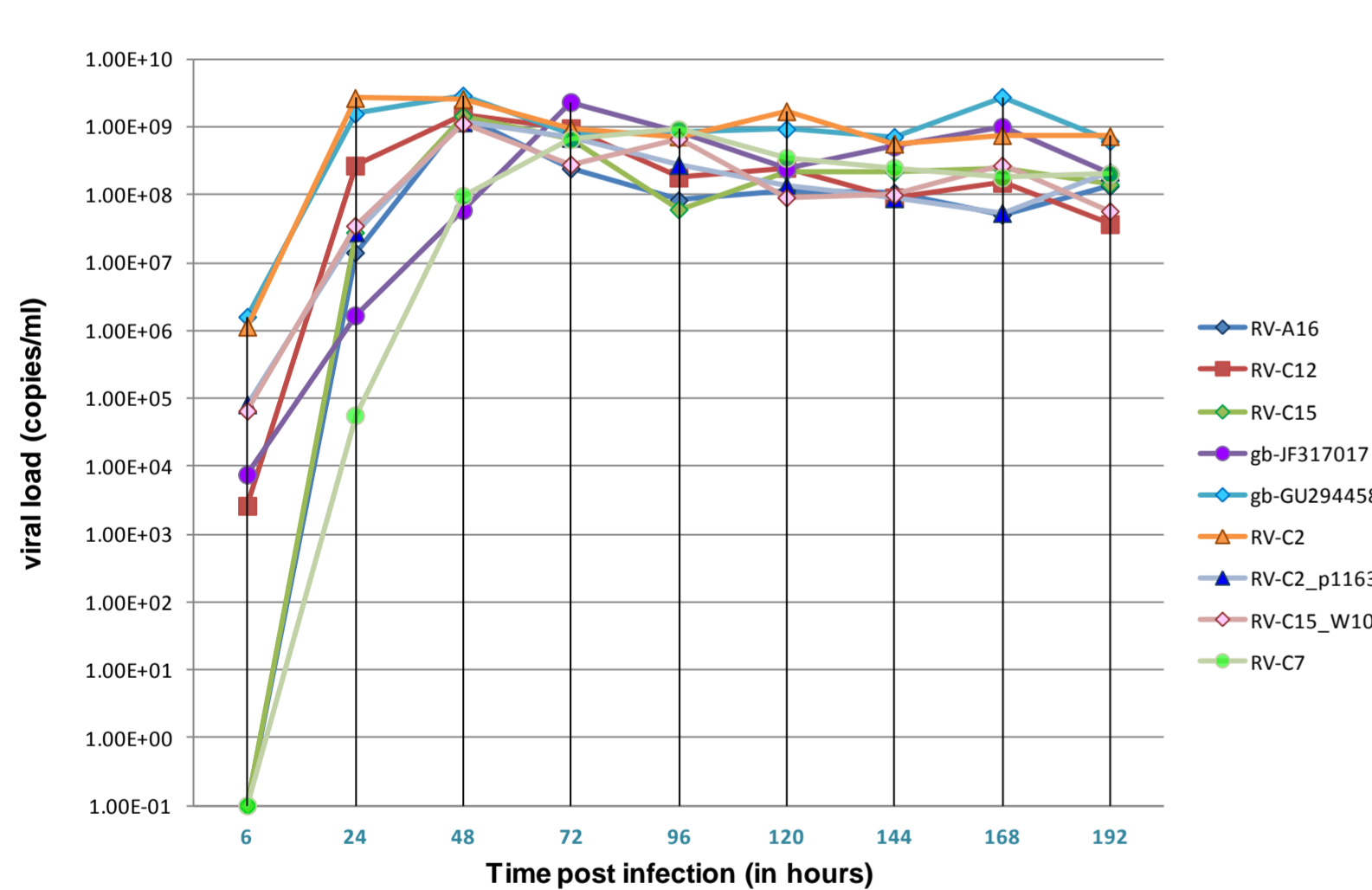


#### Protocol

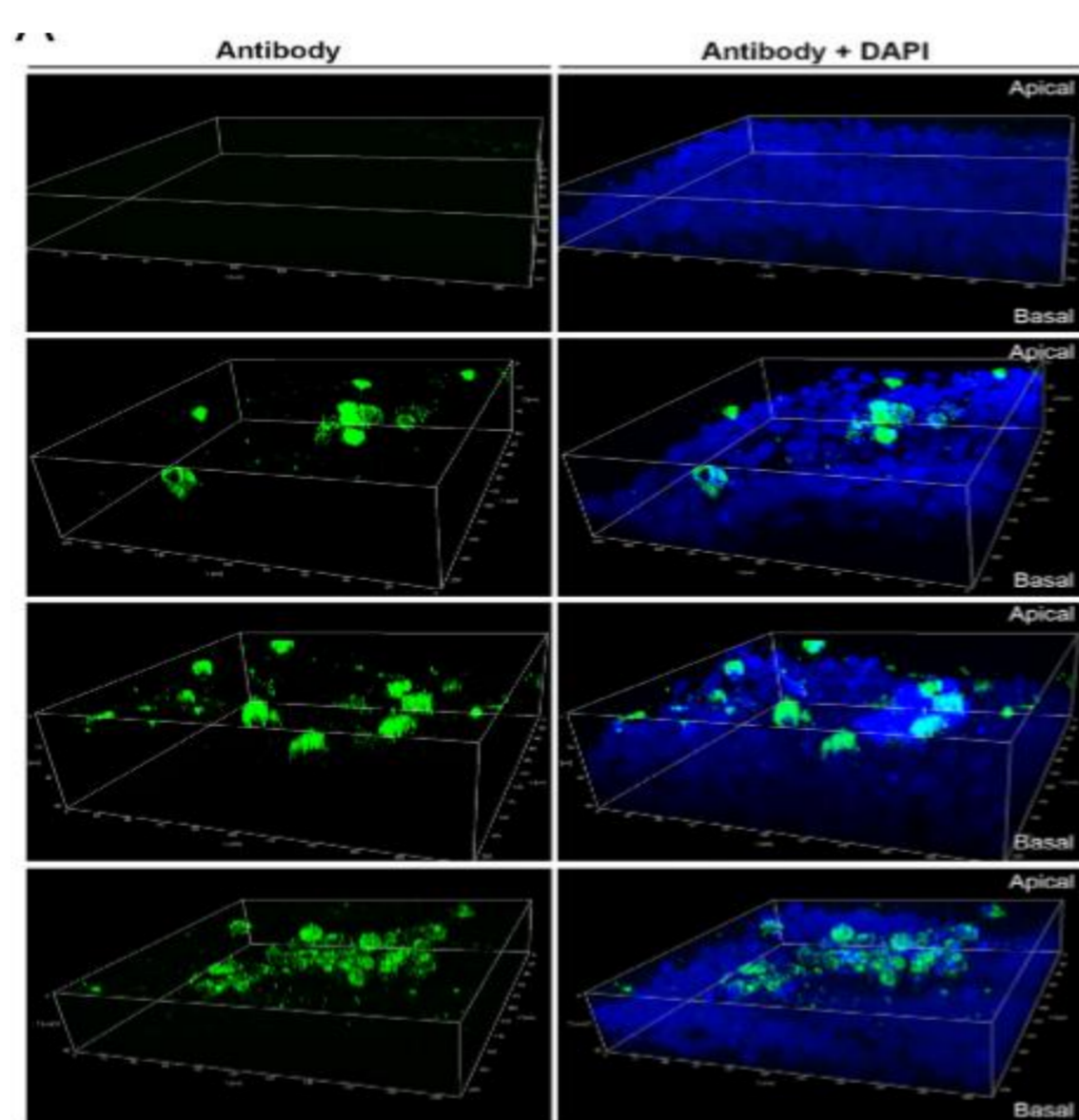
At t=0, 100 µL of inoculum was applied on the apical side of MucilAir™ for 3h30 at 34°C. Epithelia were then washed thrice with PBS in order to eliminate the unattached viruses. Apical washes were performed with 200µL of MucilAir™ culture media during 20 min at 34°C. MucilAir™ culture media was changed daily with or without a new dose of Rupintrivir (500 or 5000nM). Supernatant were lysed and viral RNA was extracted with the QIAamp® Viral RNA kit from Qiagen. Viral RNA was quantified by RT-PCR with the TaqMan ABI 7000.

### HRV-C replication in MucilAir™

#### Growth curve



#### Immunofluorescence

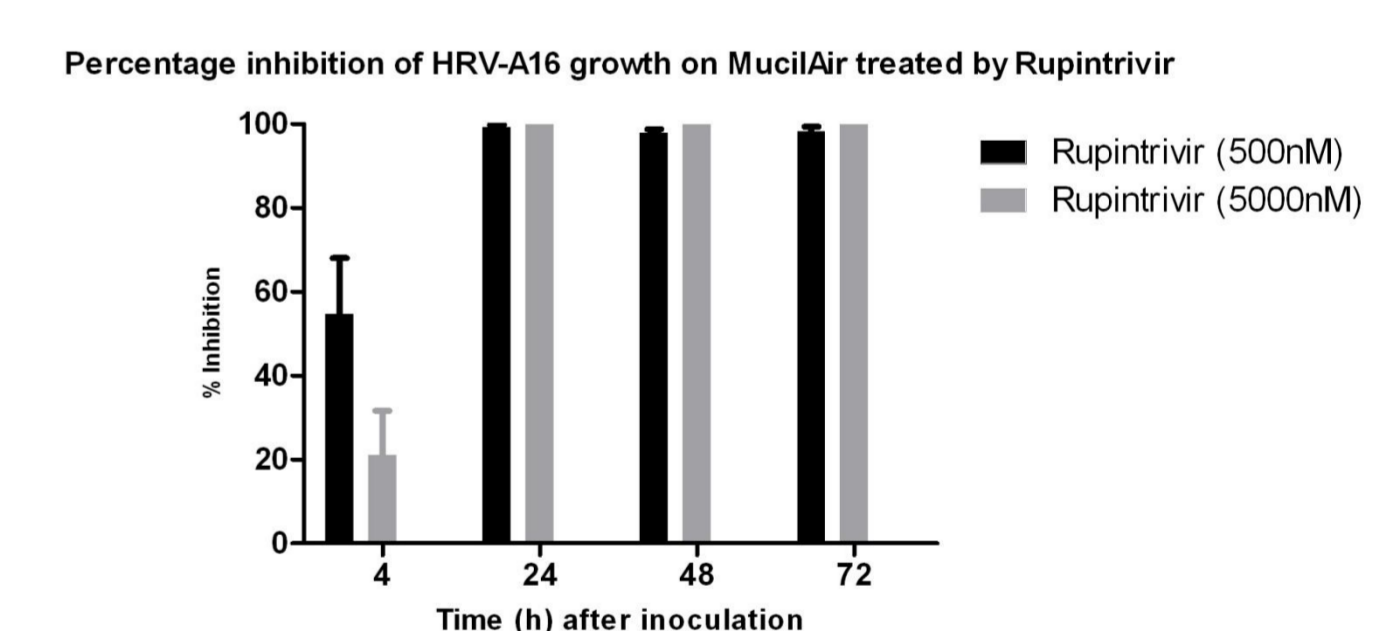
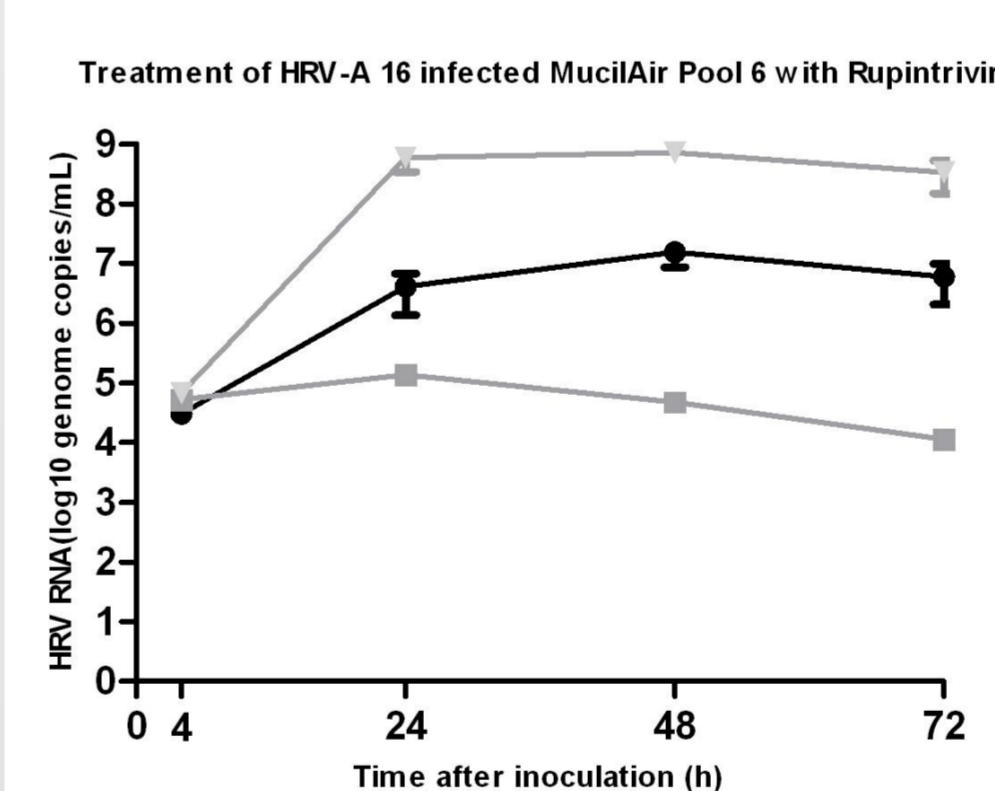


When available, the RV-C type is indicated (RV-Cxx) on the right. For untyped strains, the most similar entry in Genbank (found by blast analysis of the sequenced strain) is indicated (gb-xxxx). RV-A16 was used as a control. Viral load in cell supernatants was quantified with « Enterhino/Ge/08 » one step real-time RT-PCR. UV-irradiated clinical specimen presented undetectable ct values (not shown).

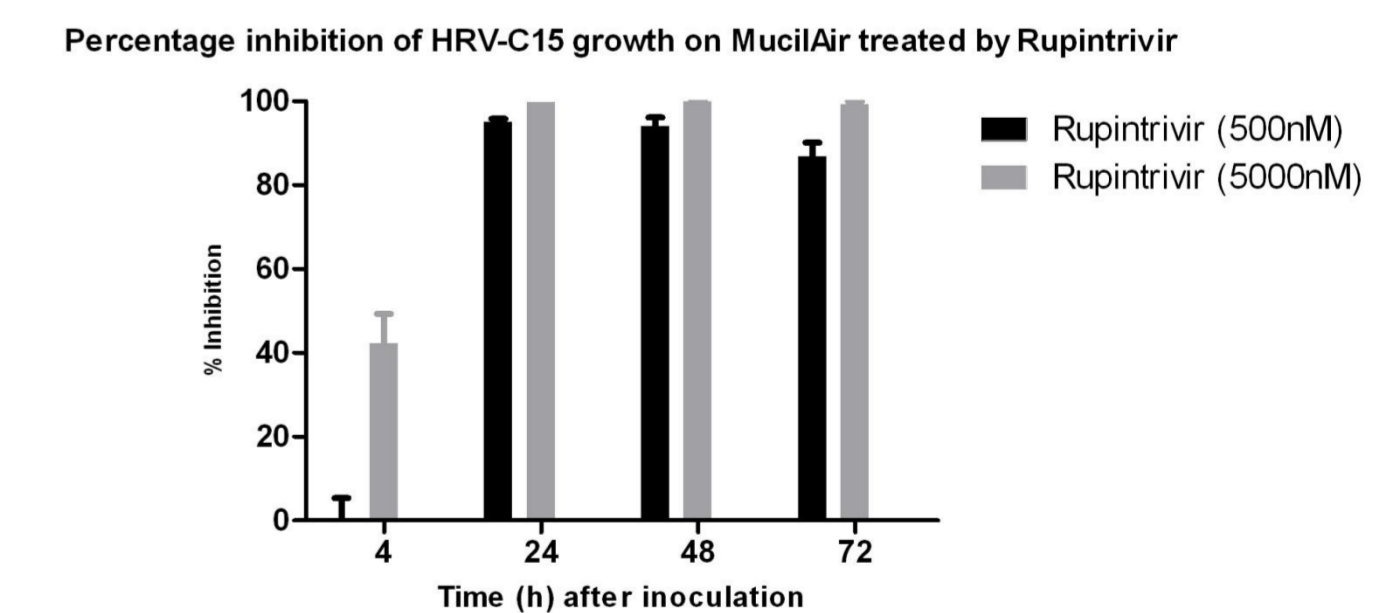
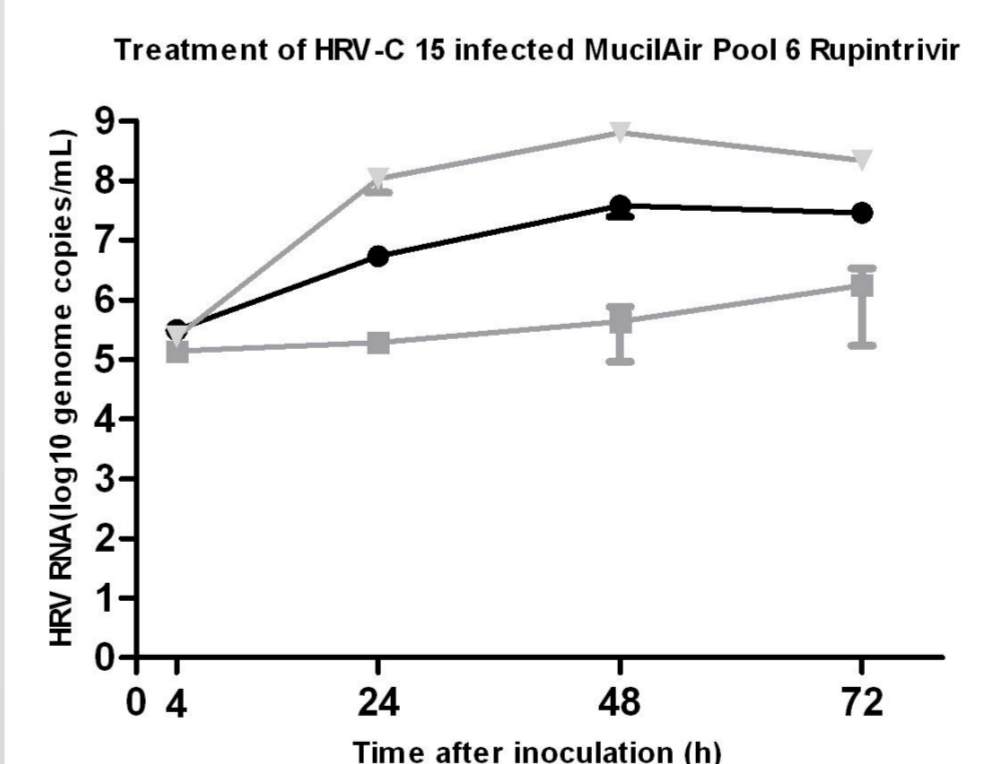
Localization of HRV infected cells in MucilAir™. Immunofluorescence with the mAbJ2 antibody detecting double stranded RNA was performed on total tissue infected for 72h with the HRV strain indicated on the right. Left column corresponds to labeling with mAbJ2 antibody. The right column represents a merge with the dapi coloration.

### Viral inhibition

#### HRV-A16



#### HRV-C15



Rupintrivir inhibited the replication of both HRV A16 and C15 in a time and dose dependent manner.

### Conclusions

### Acknowledgements

### More Information

- 1: MuciAir™ allows efficient growth of HRV-C.
- 2: The growth of HRV A16 and C15 can be blocked efficiently by Rupintrivir.
- 3: MucilAir™ is a robust, reliable and relevant tool for antiviral drug development.

