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Respiratory organoids models from bronchial cells of asthmatic patients : Fabrication and study of viral infection permissivity

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Introduction

With **4 millions people in France**, asthma is a serious public health issue. The development of new models mimicking lung tissue complexity and functionalities is essential to fight and understand this pathology.

Respiratory viruses are the first incriminated in asthma exacerbation¹ with a predominance of Respiratory syncytial viruses (RSV) and human Rhinovirus (RV) even in co-infections (30%)², but aim of viruses in asthma etiology still misunderstood

NEW MODEL : ORGANOIDS

3D multicellular tissues, aim is to mimic human organs complex functionalities

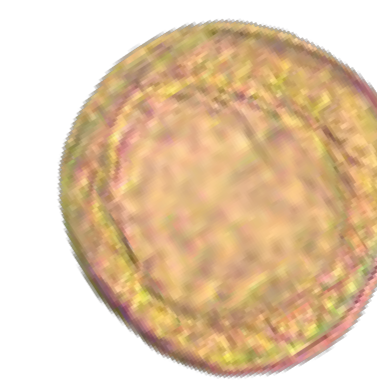
2022 : development of **healthy respiratory organoids with inverted polarity** (ciliated cells on external face, allow exposure of virus target cells) and infected with RV and Influenza³.

Goals

→ **Develop apical out organoids with bronchial cells of asthmatic patients**

Primary epithelial cells from the respiratory tract of asthmatic donors

Inverted polarity respiratory organoids



- 1) Production**
- Characterize the model
 - Confirm apical out configuration
 - Define cellular composition

Pilot study : produce preliminary data on the first asthma organoids model

Characterize Improve Criticize

2) Successful production

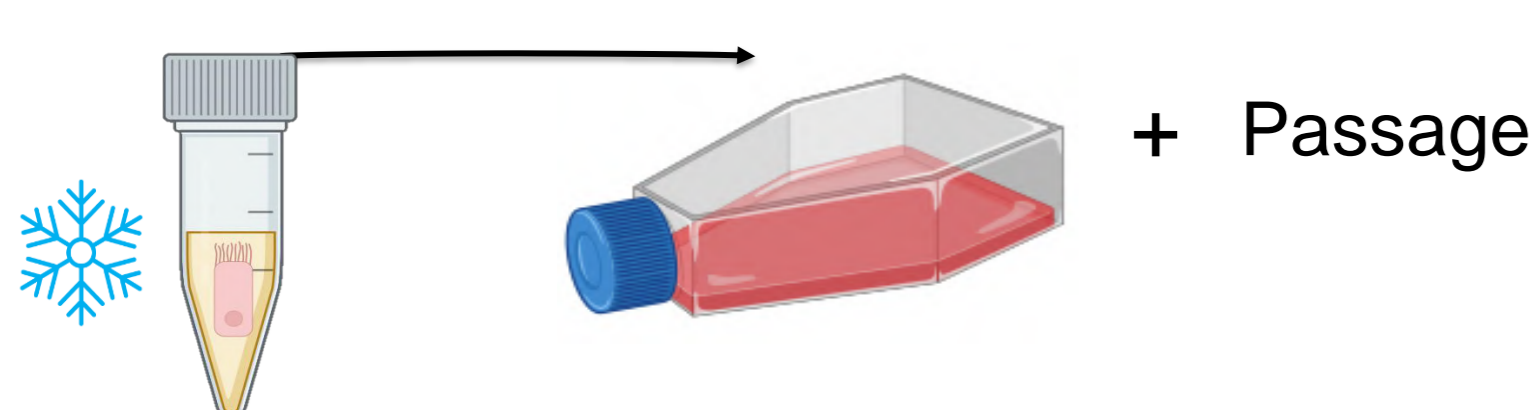
- Analyse the impact of **RSV subtype A** infection on asthmatic organoids

Compare to a control (healthy cells)

Materiels et Methods

1 Organoids production :

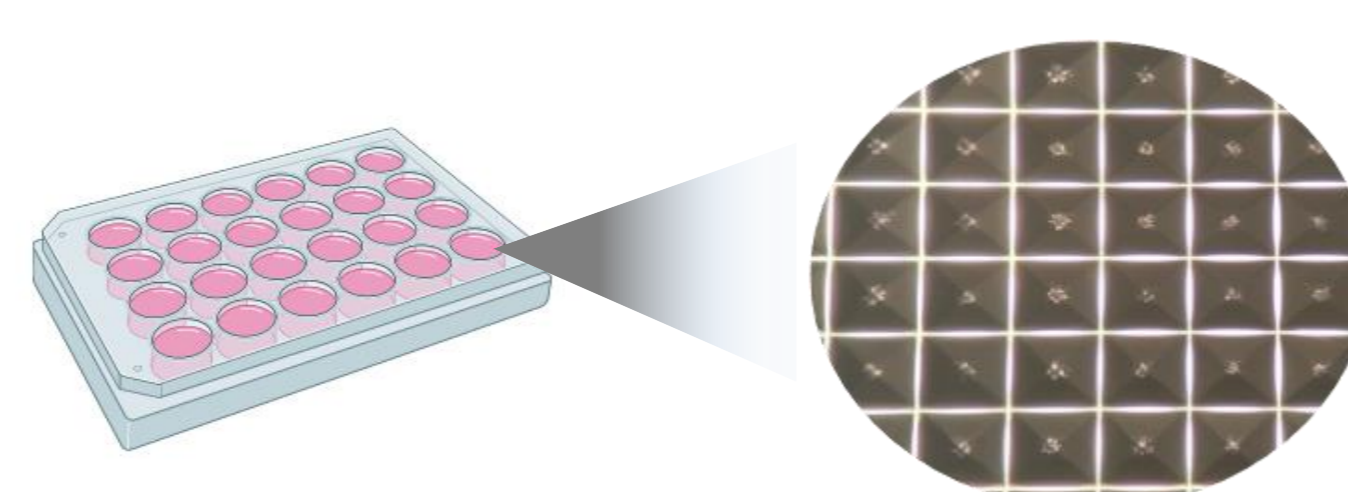
Thawing human airway epithelial cells (hAEC) (healthy +asthmatic donors) and grow in hAEC medium (Epithelix)



2D cell culture

PneumaCult™ Medium + AggreWell™ 400 plates (Stemcell)

Differentiation of hAEC cells to mature apical-out airway organoids, generates organoids in 15 days



Cellular aggregation

Fused asthma organoids at D15



***Optimization :**
Determine number of day in Aggrewell based on the model to avoid fused organoids

After aggregation time, use a flat bottom plate until D15

Organoids maturation

Immunofluorescent staining :
DAPI for nucleus
Leica Stellaris 8 (confocal microscopy)

Bioimaging PRIMACEN Plateform

2 Successful production: Infection D15



Infection : culture supernatant, 2h

After infection : 100 organoids per well (96w plate) in triplicate

Infection kinetics :
Collect at **D1, D2, D4, D6** and **D8** post infection (pi)

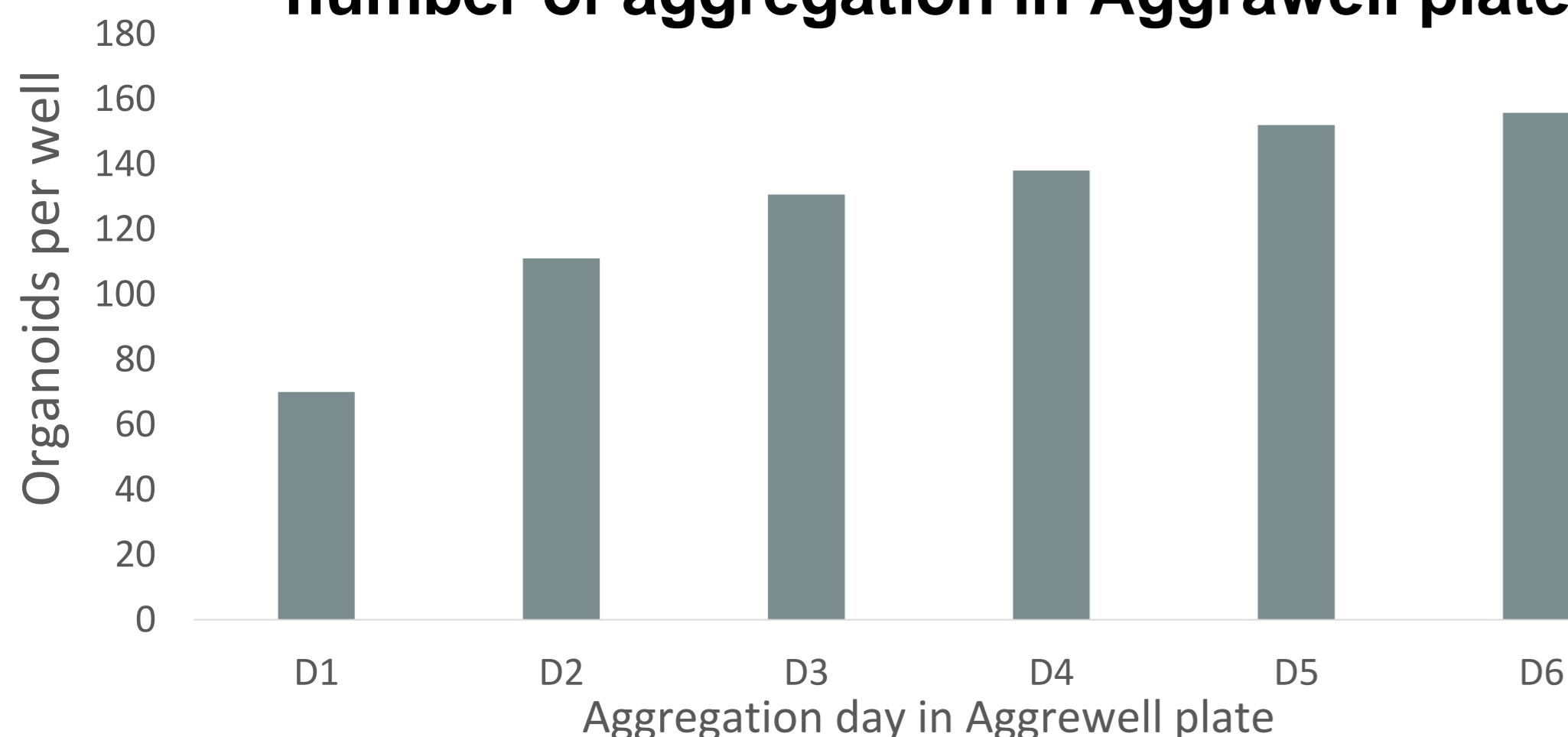
RNA Extraction :
kit EZ1 DSP virus Qiagen

Viral quantification qRT-PCR (CFX96 Biorad)

Results

1 Organoids production

1) Asthma organoids count at D15 according to number of aggregation in Aggrewell plate



→ **Day (D) 4, D5 and D6** selected (lot of fused organoids on D1, D2 and D3)
→ **D3** selected for healthy organoids

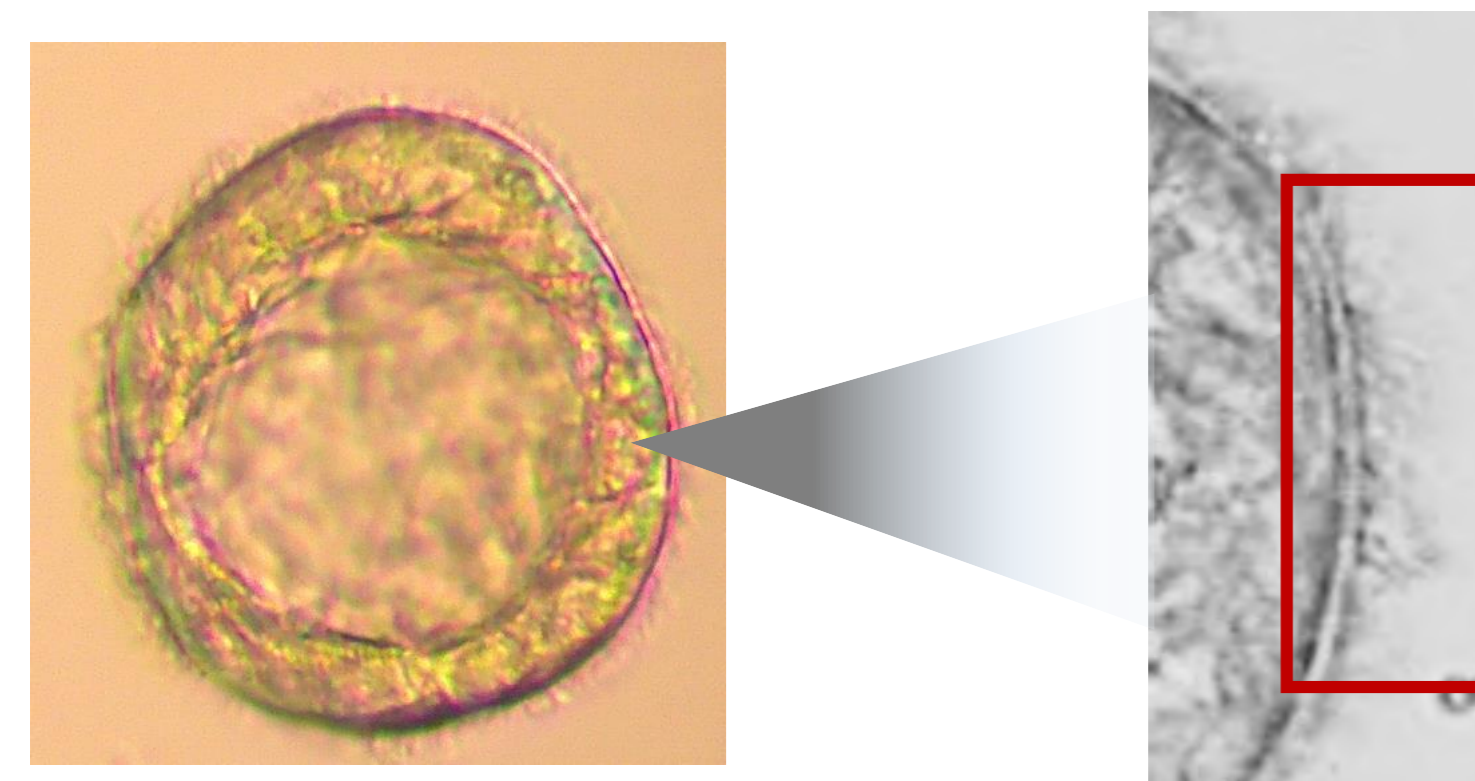
2) Organoids size

Interval of **50µM - 150 µM** for healthy and asthma models

Expected mean Diameter³ : **104.2 µM** by day 15

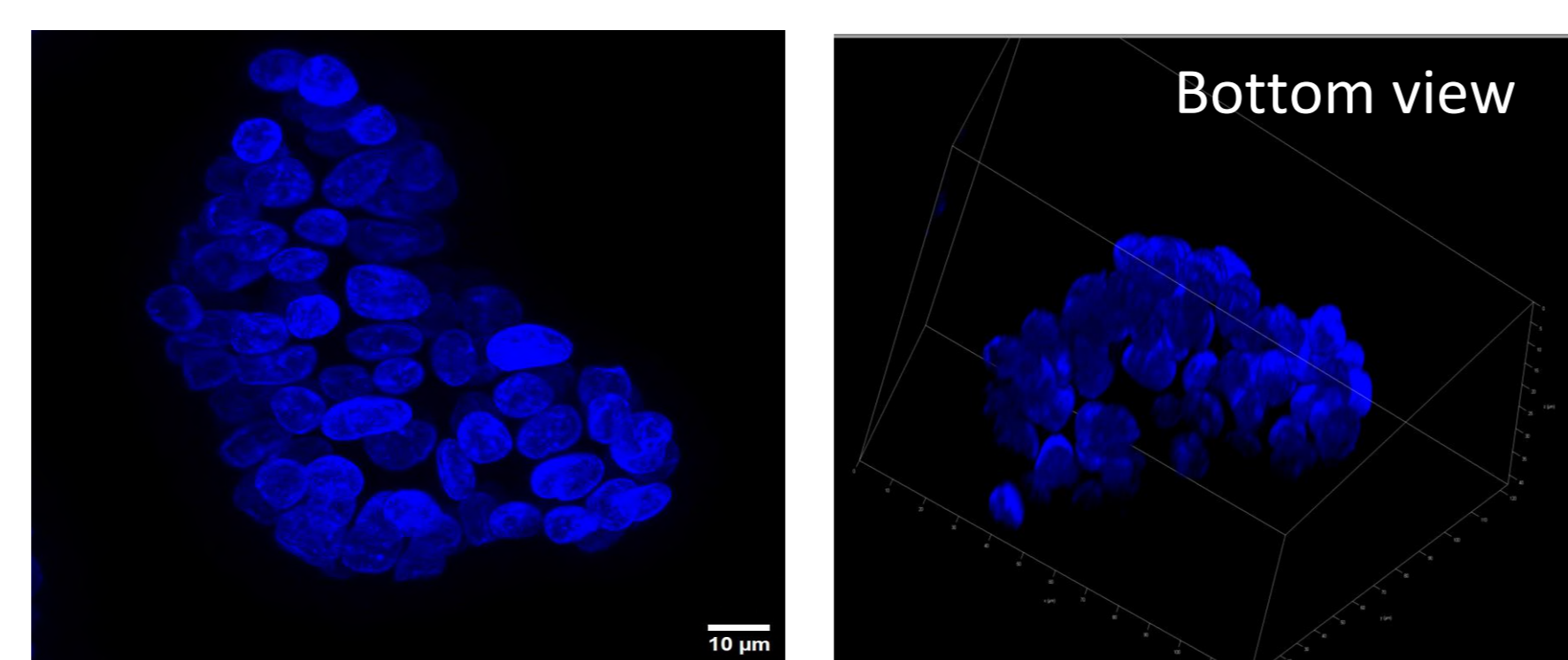
→ **Results consistent with the literature**

3) Ciliated and mobile organoids



Mobile organoids with cilium on external face (observed with inverted microscopy) : **inverted polarity confirmed**
→ **Organoids can be infected with RSV-A**

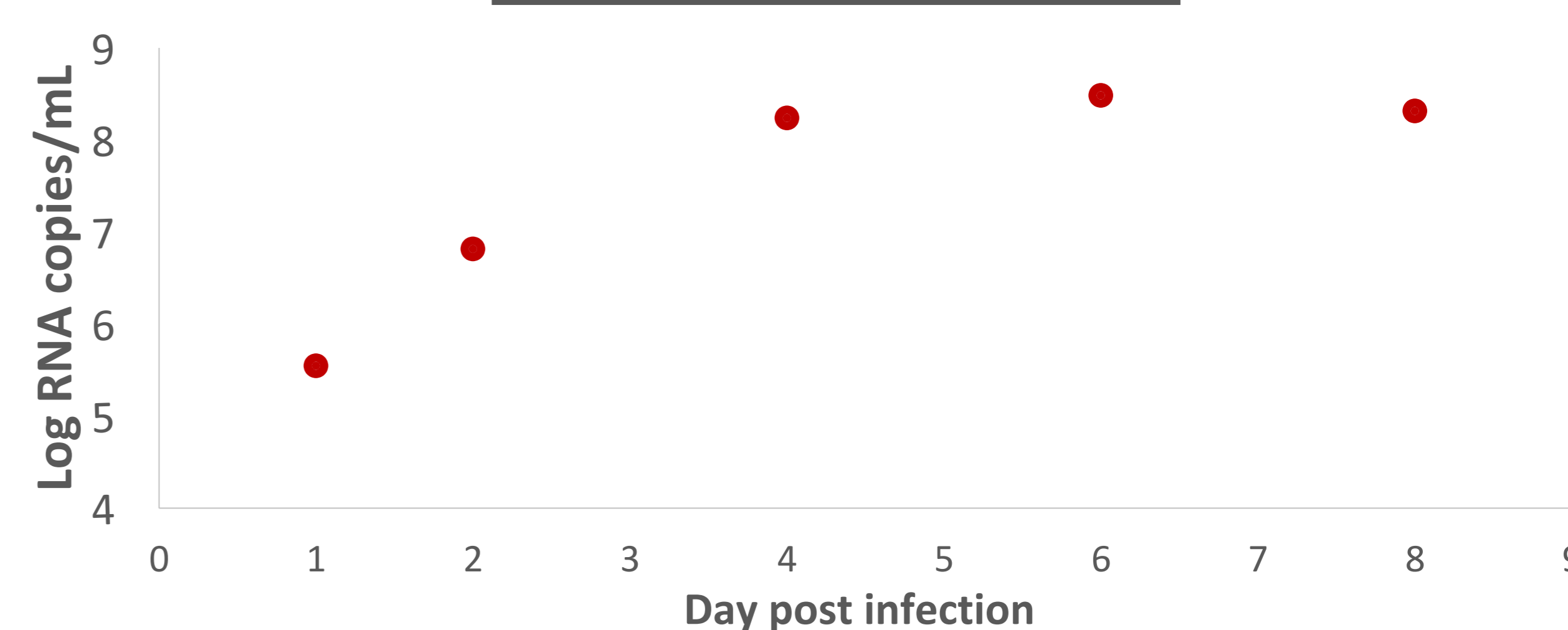
4) Immunofluorescent staining PRIMACEN collaboration



Nucleus in asthma organoid marked with DAPI : at least **61 nucleus/organoid**
→ **Limit : fluorescent signal loss (DAPI hard to reveal with Stellaris)**

2 Infection reveal : kinetics

RSV-A kinetic of infection (D1 to D8) on asthma organoids in assay 1

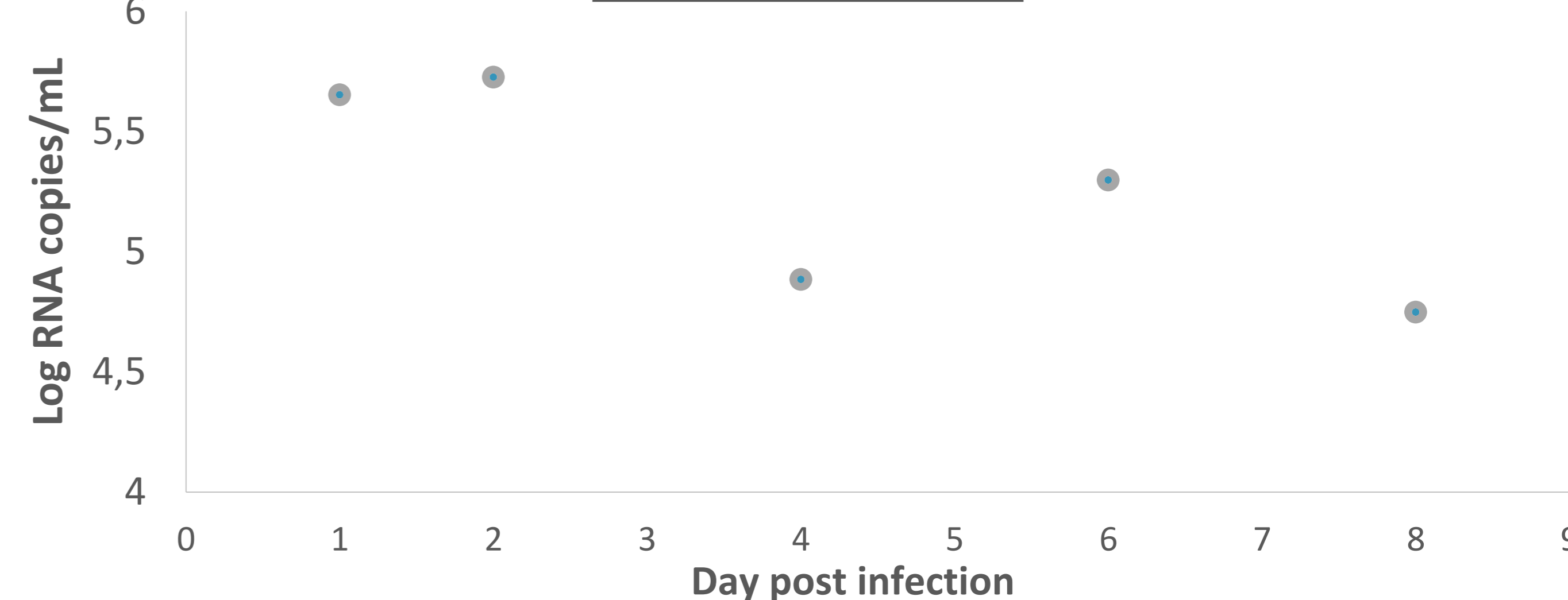


Asthma organoids :

Plateau : **1.73x10⁸ RNA copies/mL at D4** (or 8,23 log)

→ **Viral replication** for asthmatic organoids, non reproducible results in assay

RSV-A kinetic infection (D1 to D8) on healthy organoids



Healthy organoids :

7.67x10⁴ RNA copies/mL at D4 (or 4,88 log) : **NO viral replication** over the days (same results in assay 2)

Conclusions

Bibliography and Acknowledgements

1) Organoids production

- Aggregation time for healthy cells (**3 days**) and asthmatic cells (**4 at 6 days**)
- Range size (between **50µM** and **150 µM**) for non-fused organoids
- Mobile organoids with **cilium** on the external face → inverted polarity confirmed
- Immunofluorescent staining (IS) : successful with DAPI should test another microscope for others marked antibodies (Leica Thunder Tissue 3D)
- Prospect** : Check asthma phenotype with IS on the **4 main bronchial cell types**

2) Infection

- Successful asthma organoids infection with RSV : **decrease of Cq** over the days post infection for asthmatic cells but not reproducible → hypothesis : work on infectious dose
- Prospect** : test RV infection on organoids and work on **co-infection RV/RSV**

¹ Jackson D. J. *et al* (2014). IL-33-Dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo.

² Petat H *et al* (2021). High Frequency of Viral Co-Detections in Acute Bronchiolitis.

³ Stroulios, G. *et al* (2022). Apical-out airway organoids as a platform for studying viral infections and screening for antiviral drugs.

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