



**HAL**  
open science

# Fabrication and Potential Use of Apical-Out Bronchial Organoids from Asthmatic Patients for Viral Infection Studies

Flora Archambault, Fabienne Tombette, Magalie Benard, Ludovic Galas, Caroline Tapparel, Samuel Constant, Meriadeg Ar Gouil, Jean-Christophe Plantier, Elodie Alessandri-Gradt

► **To cite this version:**

Flora Archambault, Fabienne Tombette, Magalie Benard, Ludovic Galas, Caroline Tapparel, et al.. Fabrication and Potential Use of Apical-Out Bronchial Organoids from Asthmatic Patients for Viral Infection Studies. Journée Normande de Recherche Biomédicale, Jun 2024, Rouen, France. hal-04669511

**HAL Id: hal-04669511**

**<https://normandie-univ.hal.science/hal-04669511v1>**

Submitted on 8 Aug 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Flora Archambault<sup>1</sup>, Fabienne Tombette<sup>2</sup>, Magalie Benard<sup>3</sup>, Ludovic Galas<sup>3</sup>, Caroline Tapparel<sup>4</sup>, Samuel Constant<sup>5</sup>, Meriadeg Ar Gouil<sup>6</sup>, Jean-Christophe Plantier<sup>2</sup> and Elodie Alessandri-Gradt<sup>2</sup>

<sup>1</sup> Univ Rouen Normandie, Université de Caen Normandie, INSERM, Normandie Univ, DYNAMICURE UMR 1311, F-76000 Rouen, France; <sup>2</sup> Univ Rouen Normandie, Université de Caen Normandie, INSERM, Normandie Univ, DYNAMICURE UMR 1311, CHU Rouen, Department of virology, F-76000 Rouen, France; <sup>3</sup> Univ Rouen Normandie, INSERM, CNRS, Normandie Université, HeRaLeS US51 UAR2026, Primacen, F-76000 Rouen, France; <sup>4</sup> Département de Microbiologie et Médecine moléculaire, Université de Genève, 1206 Genève, Suisse; <sup>5</sup> Epithelix SAS <sup>6</sup> Université de Caen Normandie, Univ Rouen Normandie, INSERM, Normandie Univ, DYNAMICURE UMR 1311, CHU Caen, Department of virology, F-14000 Caen, France

## Introduction

With **4 millions people affected 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 exacerbation<sup>1</sup>** with a predominance of Respiratory syncytial viruses (RSV) and human Rhinovirus (RV) even in co-infections (30%)<sup>2</sup>, but aim of viruses in asthma etiology still misunderstood

### NEW MODEL : ORGANOIDS

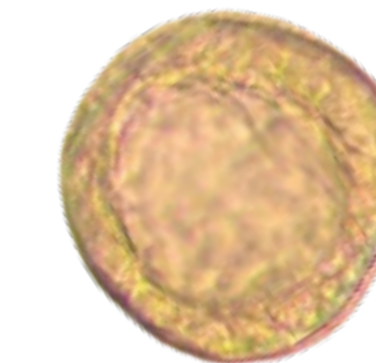
3D multicellular tissues, aim is to mimic human organs complex functionalities

**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 Influenza<sup>3</sup>.

## Goals

→ Develop apical out organoids with bronchial cells of asthmatic patients

Primary bronchial epithelial cells from asthmatic donors (provided by Epithelix)



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

Pilot study : produce preliminary data

Characterize Improve Criticize

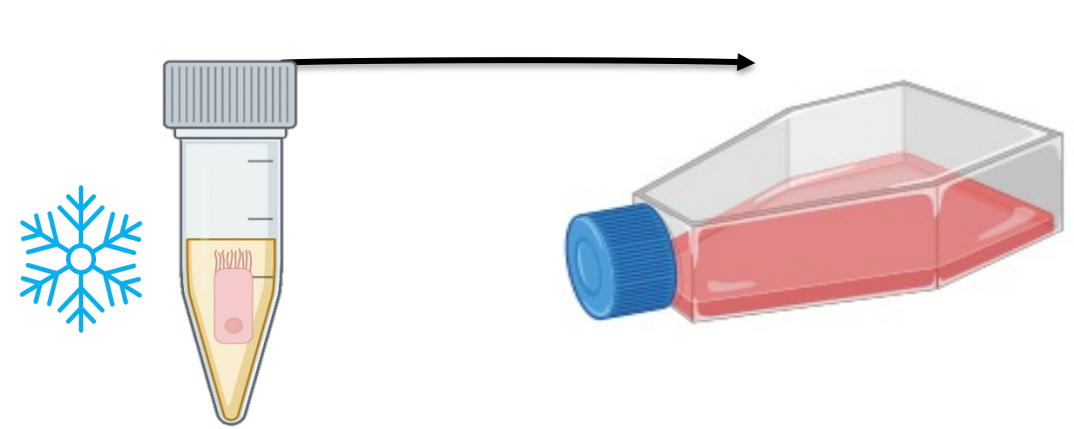
- 2) Successful production
- Analyse the impact of **A-RSV** infection on asthmatic organoids

Compare to a control (healthy cells)

## Materials and methods

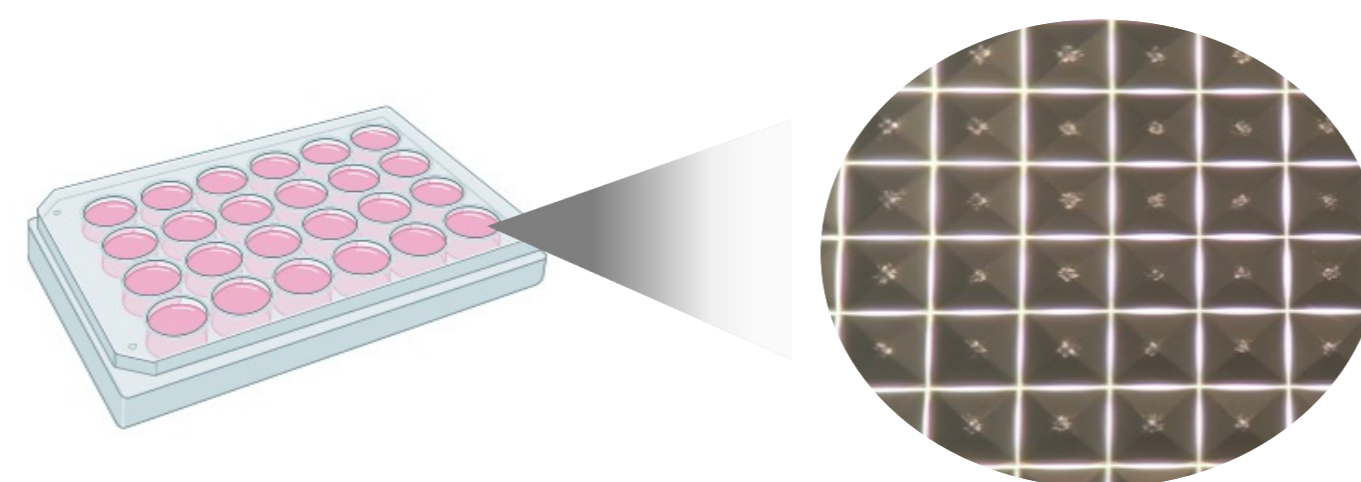
### 1 Organoids production :

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



2D cell culture

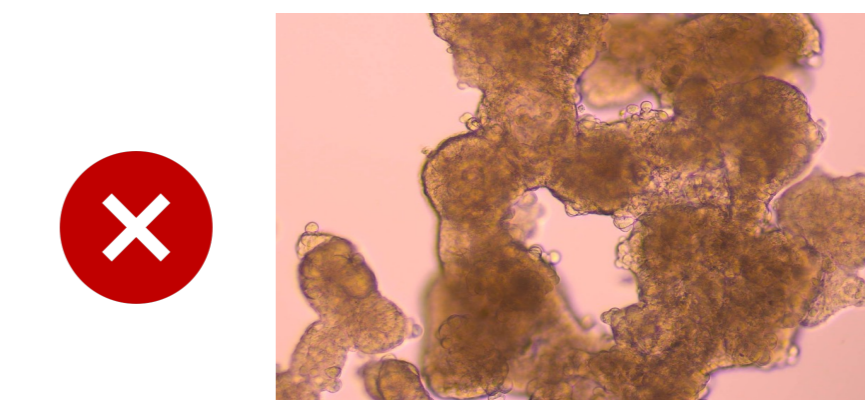
Differentiation of hAEC cells to mature apical-out airway organoids, mature organoids obtained in 15 days



Cellular aggregation

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

Fused asthma organoids at D15



After aggregation time, use a flat bottom plate until D15

Organoids maturation

Immunofluorescent staining :

DAPI for nucleus  
 Leica Stellaris 8 (confocal microscopy)

PRIMACEN Bloimaging Platform

### 2 Production successful : Infection D15

A-RSV 37°C  
 Infection : culture supernatant, 2h

After infection : 96 well plate in Triplicate : 100 organoids per well

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

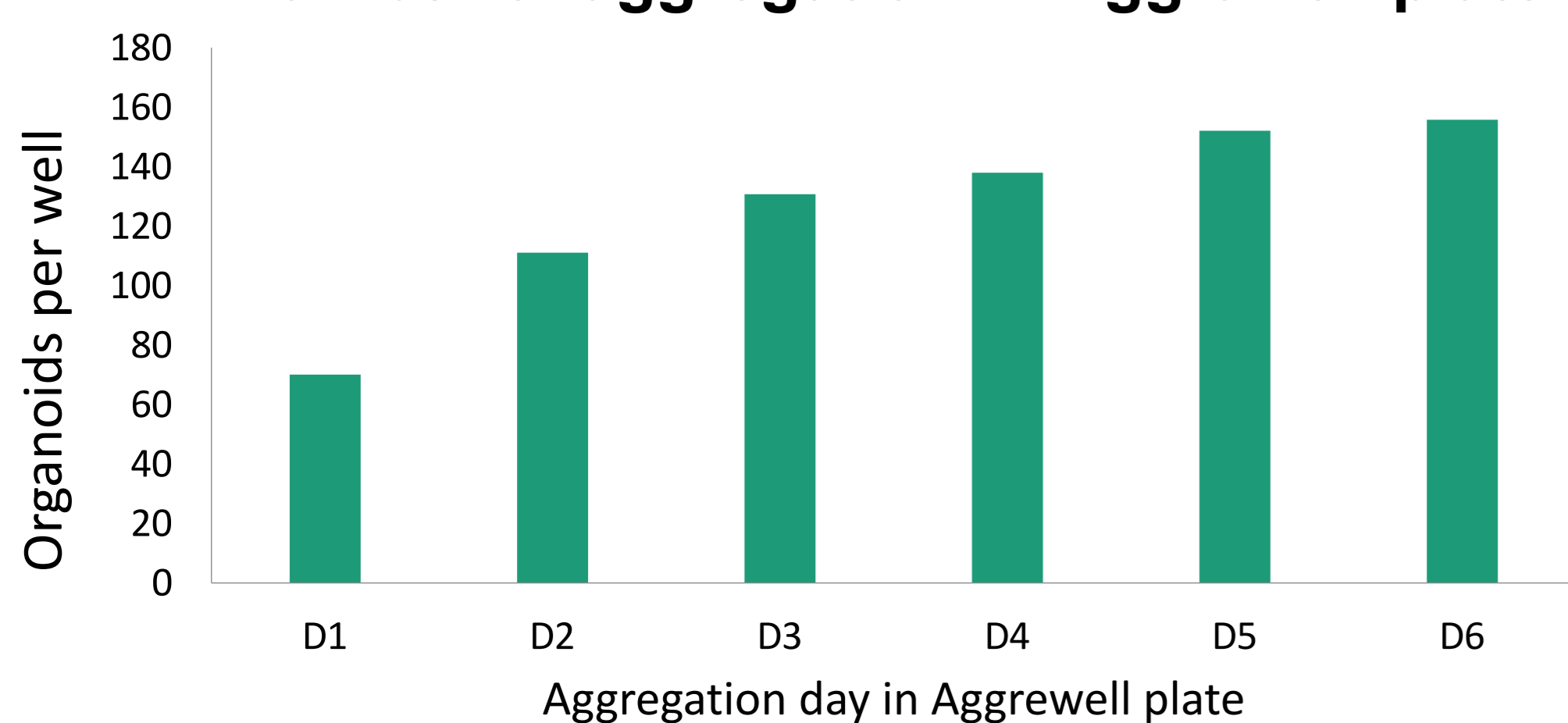
RNA Extraction : kit EZ1 DSP virus Qiagen

Viral quantification qRT-PCR (CFX96 Biorad)

## Results

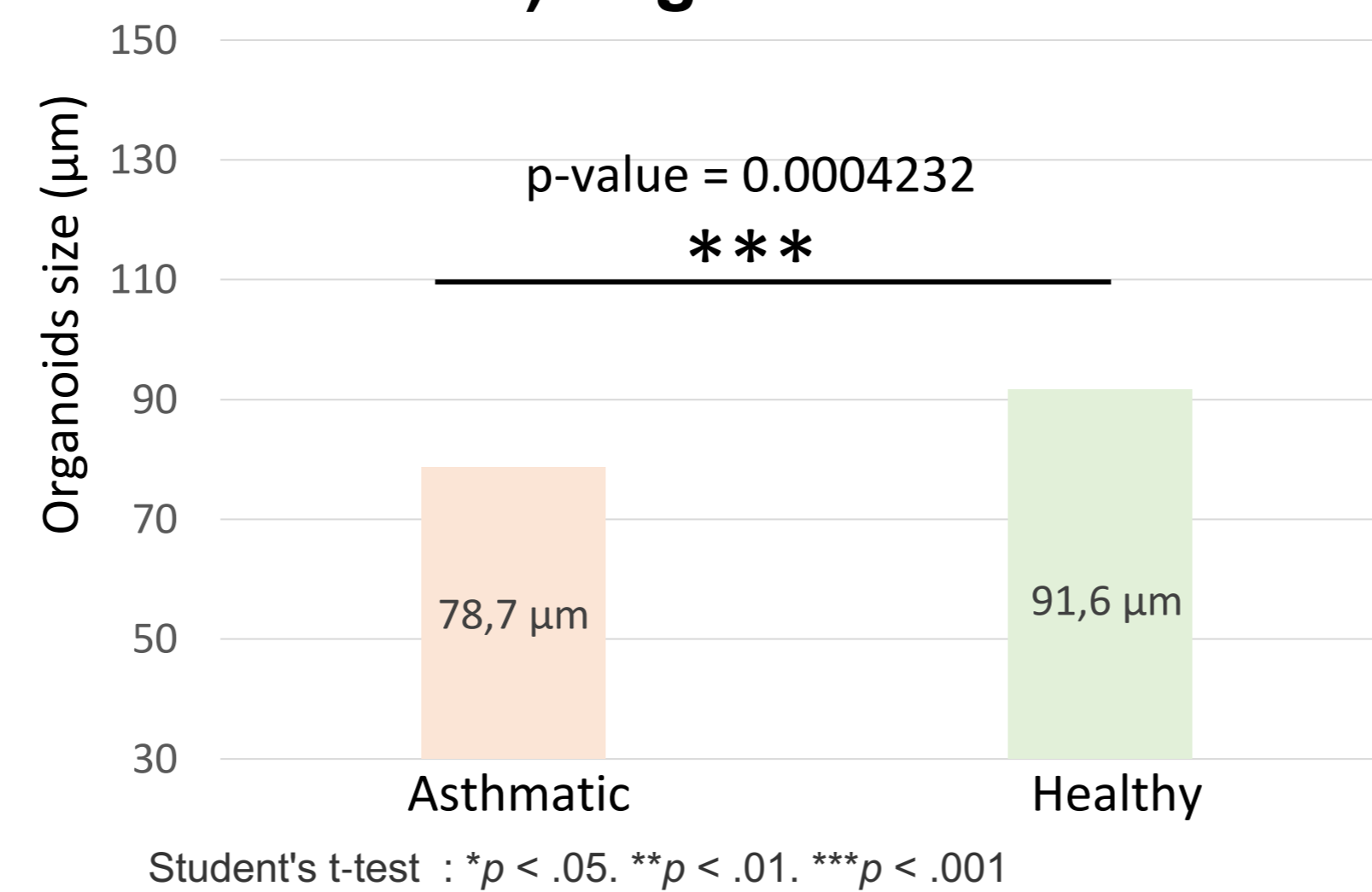
### 1 Organoids production

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



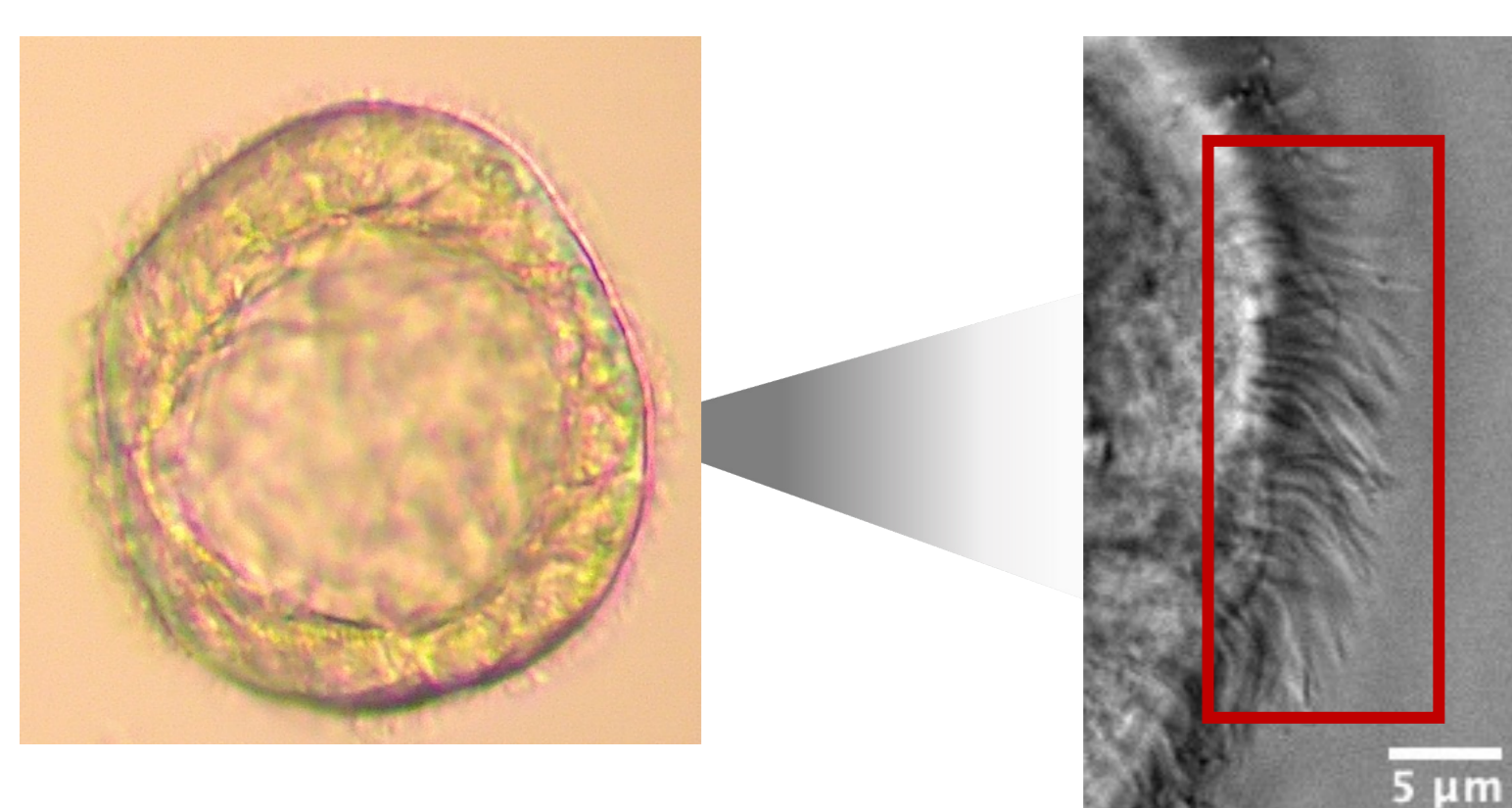
→ Day (D) 4, D5 and D6 selected (lot of fused organoids on D1, D2 and D3)  
 → D3 selected for healthy organoids

#### 2) Organoids size



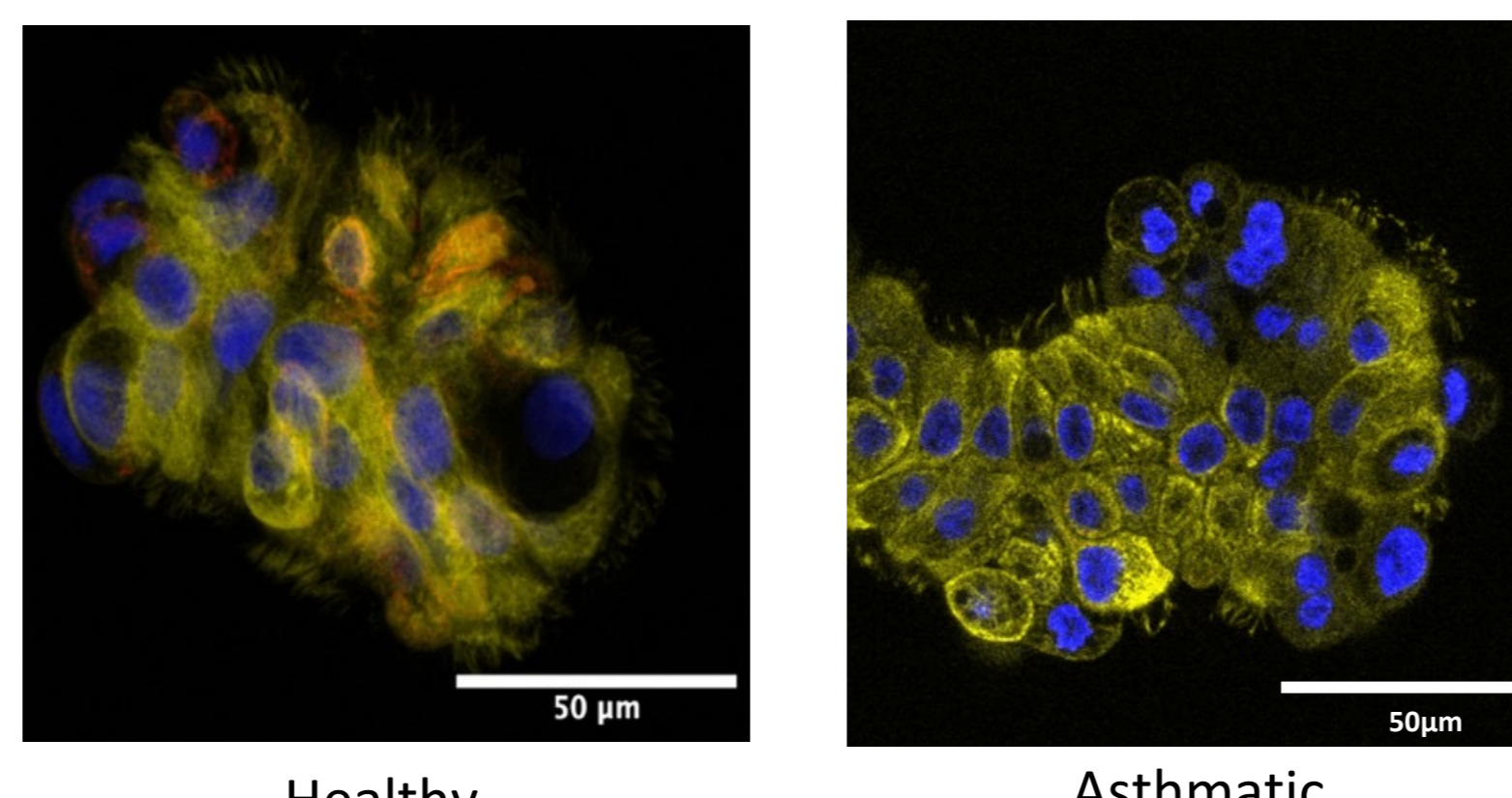
Expected mean Diameter<sup>3</sup> : 104.2 µm by day 15  
 → Asthma organoids size < healthy organoids but first results look consistent with the literature

#### 3) Ciliated and mobile organoids



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

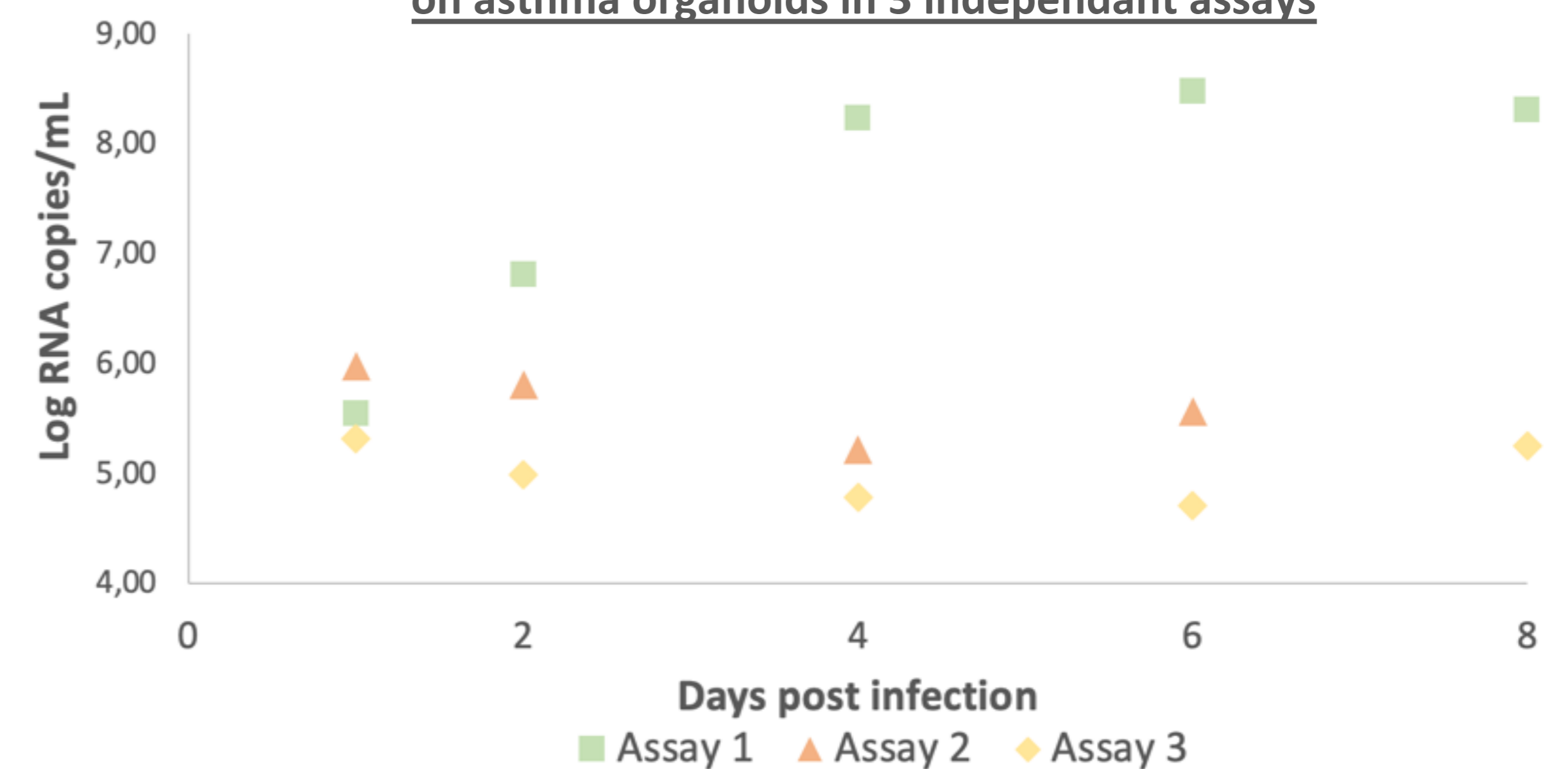
#### 4) Immunofluorescent marking



Abundance of **ciliated cells** (yellow : beta tubulin IV) Maybe more **basal cells** (red : cytokeratine 5) in healthy organoids but weak signal  
 Average of 100 cells/organoids (blue : nucleus DAPI)

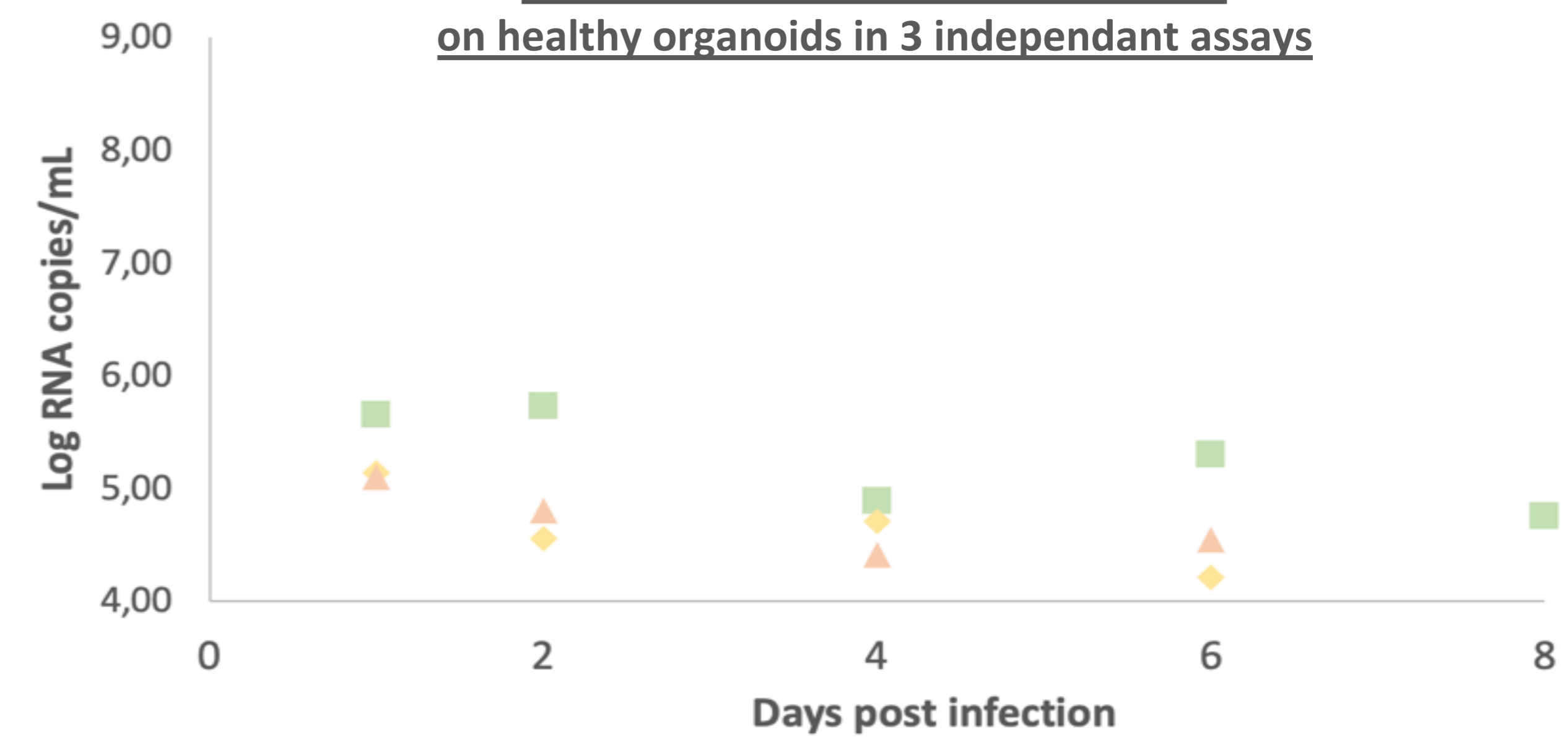
### 2 Infection reveal kinetics

#### A-RSV kinetic of infection (D1 to D8) on asthma organoids in 3 independant assays



**Asthma organoids :**  
 Plateau : **1.73x10<sup>8</sup> RNA cp/mL (8,23 Log) between D4 and D8**  
 → **Viral replication** only for assay 1, no reproducible results

#### A-RSV kinetic of infection (D1 to D8) on healthy organoids in 3 independant assays



**Healthy organoids :**  
 7.67x10<sup>4</sup> RNA copies/mL at D4 (or 4,88 log) : **NO viral replication** over the days for the 3 assays

## Conclusions

The production of reversed polarity organoids from bronchial cells of asthmatic patients has been a success. The results will be used for larger projects such as the development of **personalized medicine**. Organoids will be able to play a leading role in comparison to previous models (2D culture, ALI, animal experimentation) due to the **faithful reconstitution of tissue in vivo**.

The infection with A-RSV on asthma organoids is successful but result are not reproducible → hypothesis : work on infectious dose and ratio virus/number of organoids. RV infection on organoids and **co-infection RV/RSV** are in progress on this model

→ Need to improve model robustness by **adding immune** cells and work on infection **reproducibility**

## Bibliography and Acknowledgements

- Jackson D. J. *et al* (2014). IL-33-Dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo.
  - Petat H *et al* (2021). High Frequency of Viral Co-Detections in Acute Bronchiolitis.
  - Stroulios, G. *et al* (2022). Apical-out airway organoids as a platform for studying viral infections and screening for antiviral drugs.
- Acknowledgment :** Dr Sophie Candon for her wise advices about immunology and Stéphane Rosset for his rapidity and help about RT-qPCR.