Miniaturized primary airway epithelia and reporter viruses development to screen antiviral molecules against respiratory viruses

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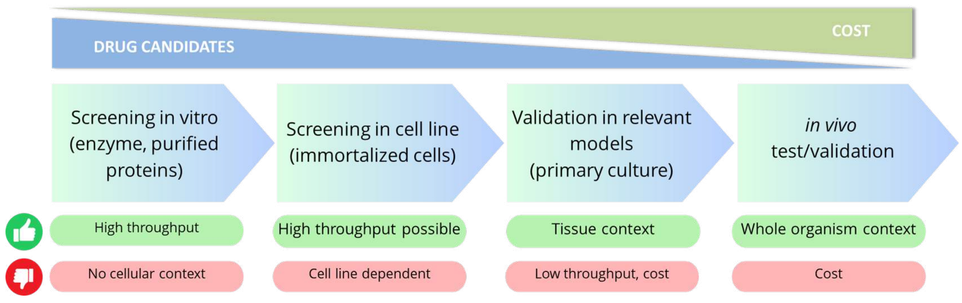
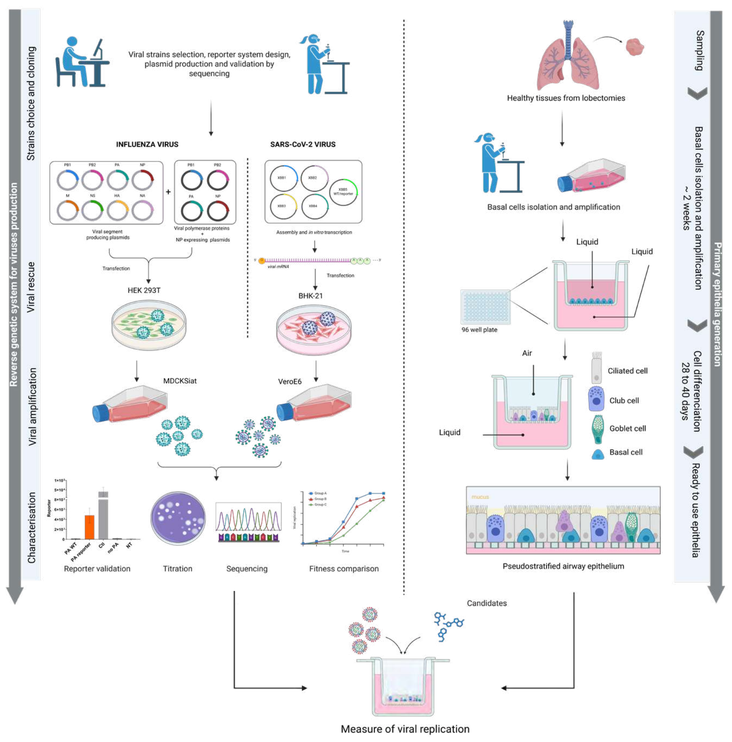
Introduction

Discovery of new antiviral drugs against respiratory viruses remains limited. The COVID-19 pandemic exposed the limitations of current screening methods, which mostly rely on immortalized cell lines and lab-adapted viral strains—failing to reflect the proper infection of human tissues. To address these issues, we are developing a more physiologically relevant screening platform using primary human airway epithelia in a 96-well air-liquid interface (ALI) format, combined with recent influenza and SARS-CoV-2 unique reporter strains. This system enables rapid, real-time, and cost-effective drug screening and evaluation. We believe that the use of a more relevant cellular model should improve the quality of results, increase the success rate in vivo and therefore reduce the number of animals required, in line with the 3Rs principle.

1 Drug candidate identification and validation steps

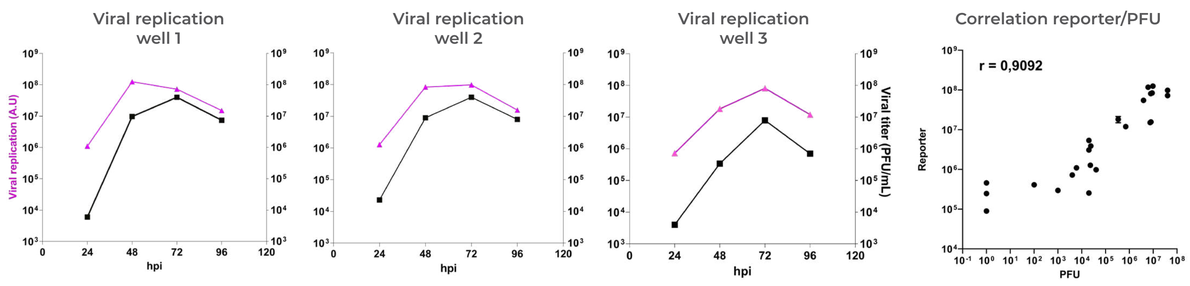


2 System set-up and readout

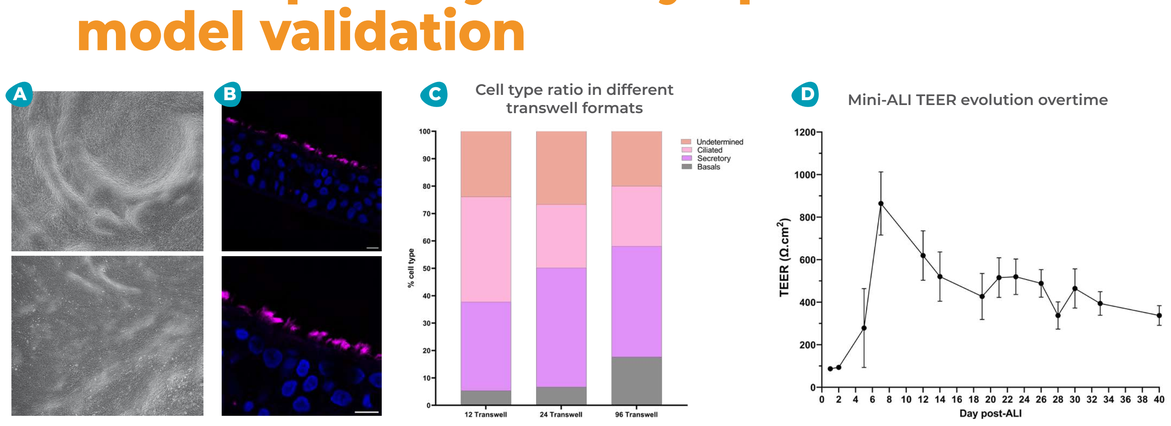


	Classical titration	RTFS System
Time	> 8 days	Immediate
Scale	Limited	High
Cost	High	Low

RTFS REAL-TIME FLU SCREEN



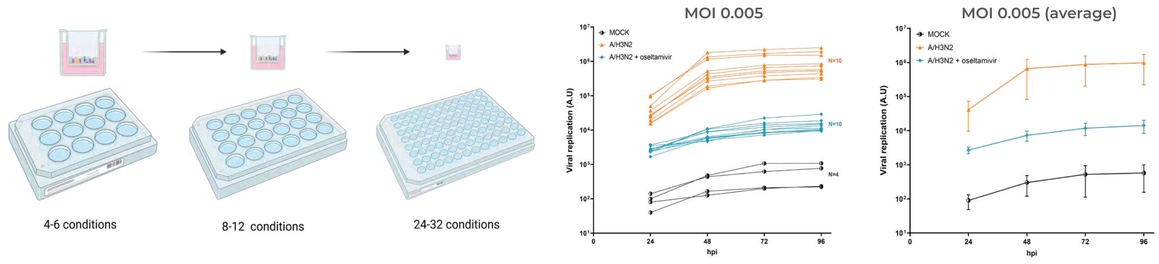
3 Human primary airway epithelium model validation



Those characterizations allow us to assess the quality of the epithelia produced from every donor we biobank. We check cilia beating, mucus production, pseudostratified structure and the proper proportions of the different cell types in these epithelia. The TEER measurements also allow verification of the quality of the differentiation.

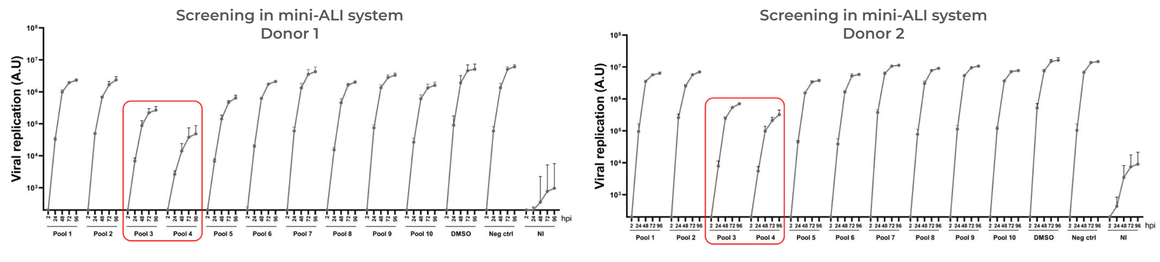
(A) Brightfield microscopy showing mucus accumulation on the top of pseudostratified epithelia in ALI condition
(B) Confocal microscopy on histological sections of pseudostratified epithelia on transwell. Tubulin (magenta), nuclei (blue)
(C) Cell type ratio measurements by flow cytometry and comparison between different transwell sizes.
(D) Mini-ALI TEER measurement overtime to assess the epithelia differenciation level
(E) Histological cut and Hematoxylin & Eosin coloration of a differenciated epithelium (24 WP transwell)

4 System miniaturization in 96 well plate format

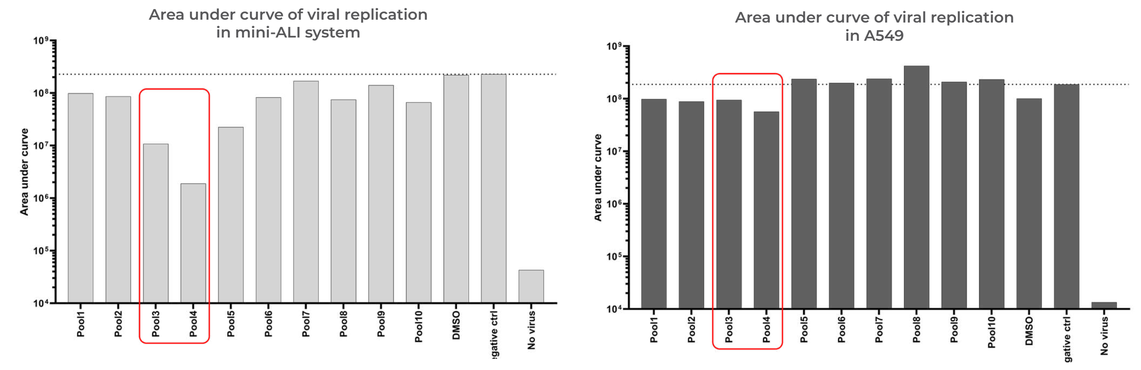


- Miniaturizing the system allows to test more molecules at the same time
- Real-time assessment of viral replication (MOI : multiplicity of infection)
- Good consistency between the wells. 3 to 4 wells are enough for statistical significance (0.27 > Z' > 0,47).

5 Compound screening in mini-ALI system and comparison with A549 cell line



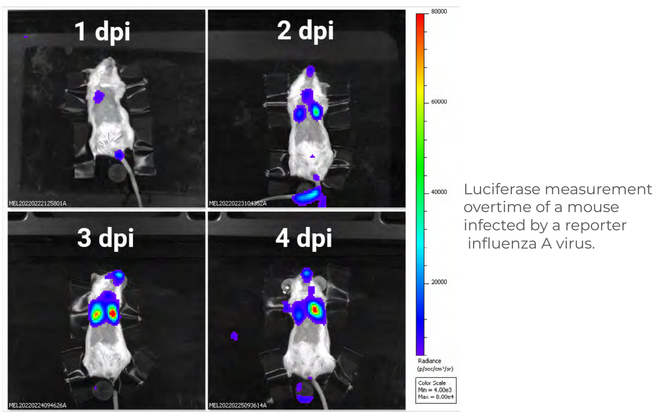
Proof of concept of a screen in mini-ALI against influenza A virus. Pools of molecules can be tested to increase even more the number of tested candidates. Viral replication is monitored overtime from the same well. Each condition was tested in duplicate for each donnor.



Comparison of drug efficacy in mini-ALI and A549 cells. The left panel represents the area under curve of infection (mean of the 2 donnors) in mini-ALI. The right pannel represents the area under curve of infection in A459 cells

6 *In vivo* testing of antiviral hits

Following-up the tests in primary mini-ALI system, hit candidates can be tested *in vivo* in ERBC Dommartin's facility.



Luciferase measurement overtime of a mouse infected by a reporter influenza A virus.

Conclusion

By integrating the ALI (Air-Liquid Interface) system and recent viral strains into our advanced drug screening service, we aim to enhance the quality and relevance of antiviral hits. This approach reduces both the duration and cost of screenings. It also reduces the selection of false positives and false negatives. The use of up-to-date viral strains ensures that candidate drugs target current circulating viruses effectively. Additionally, miniaturizing the ALI system and combining it with real-time screening technology allow us to test numerous drugs simultaneously, overcoming traditional technical limitations and bottlenecks of ALI culture. As experts in in vivo experimentation, we can also validate the hits identified in ALI screenings directly in animal models.