Bridging Airway Epithelial Cell Model and Human Challenge Studies

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INTRODUCTION

Airway epithelial cell differentiation in Air–Liquid Interface (ALI) condition closely mimics physiological environment of the human airway system, promoting enhanced oxygen availability and cellular differentiation. These conditions are valuable for toxicity screening, drug transport studies, and exploring molecular pathways. Human Challenge Studies are pivotal in understanding interactions between viruses and the human body, leading to discoveries of immune mechanism and providing key outcomes about promising investigational medicinal products (mechanism of action, most appropriate dose and dosage regimen), which can then be applied to designing Phase IIb and Phase III studies.
Here we link ALI to Human challenge studies, results from ALI will help guide the dose to be used in human challenge and determine the best timing for sampling.

AIR–LIQUID INTERFACE MODEL

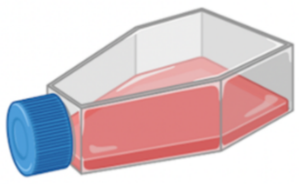
1) Nasal Brush samples are cultured immediately with antibiotics, dislodging cells and washing mucus & immune cells.
When 80-90% Confluency is achieved, cells are split and/or frozen.

2) Commercially accessible normal human airway epithelial cells can also be used.

Normal human bronchial epithelia are cultured in a collagen-coated 12-well plate, achieving 80-90% confluency over 1-2 weeks in a humidified incubator with 5% CO2.

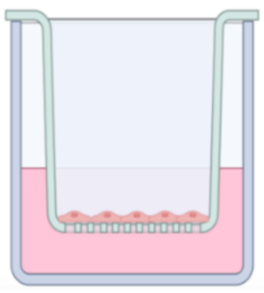
Expansion Phase

Cells are transferred to a collagen-coated T25 flask and allowed to grow again to 80-90% confluency (1-2 weeks).

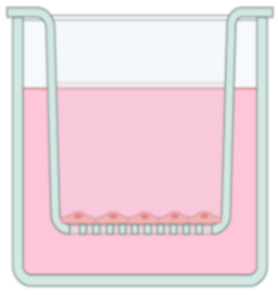


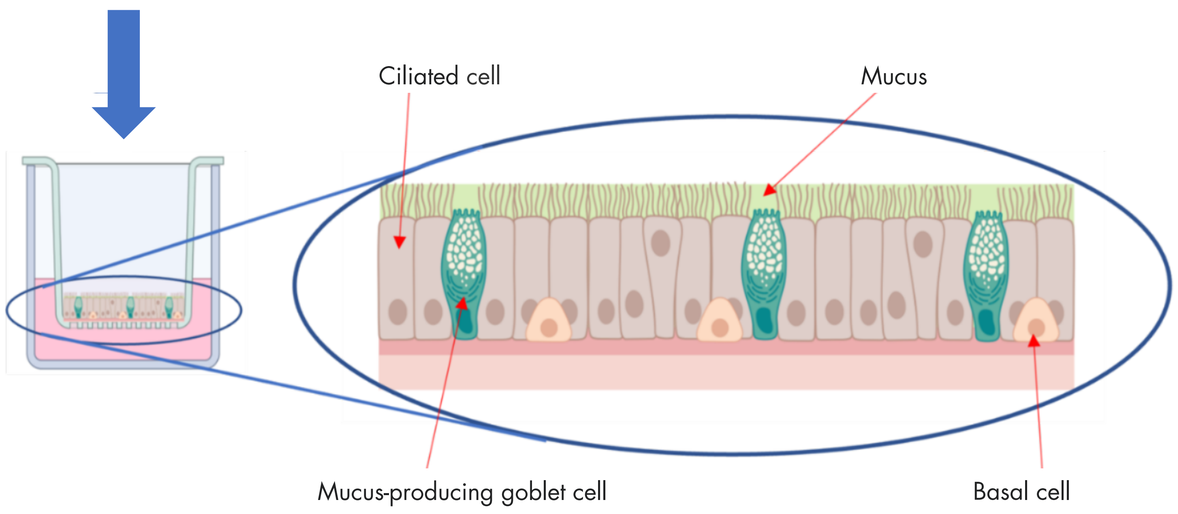
When the cells are confluent (1 week), the basal medium is changed to differentiation and the apical medium removed so the cells are exposed to air.
(1-2 weeks). This configuration mimics the conditions found in the human airway and drives differentiation towards a mucociliary phenotype.

Cells are then seeded onto the permeable membrane of a cell culture insert.



Differentiation Phase

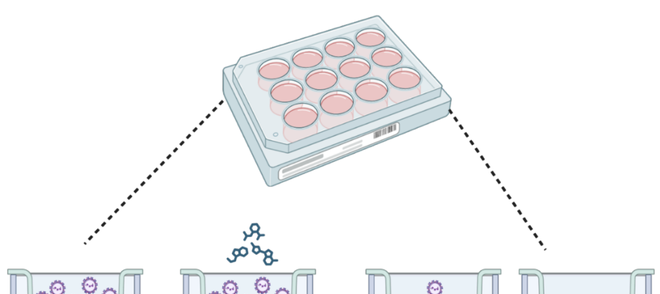




After 1-2 weeks, the cell surface became opaque and glossy, indicating that the cells are differentiating. Mucus-producing goblet cells are growing. At around 2-3 weeks after mucus appears, cilia are noted. Once cilia are first seen, the cells are expected to be ready for infection approximately 2-3 weeks later.

Figure 1: Experimental protocol for the culture of airway epithelium cells from either direct airway sampling or from commercially available starting material. Expansion and differentiation phase take approximately 8 weeks in total.

AIR–LIQUID INTERFACE INFECTION



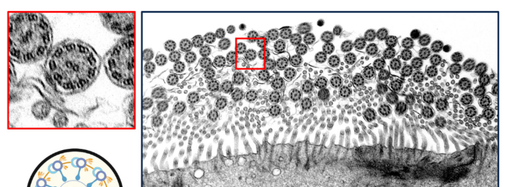


Figure 2: TEM image of in vitro ALI-cultured cilia in transverse section, showing a "9 + 2" microtubular arrangement.
Pictured by Dr Andrew Rogers

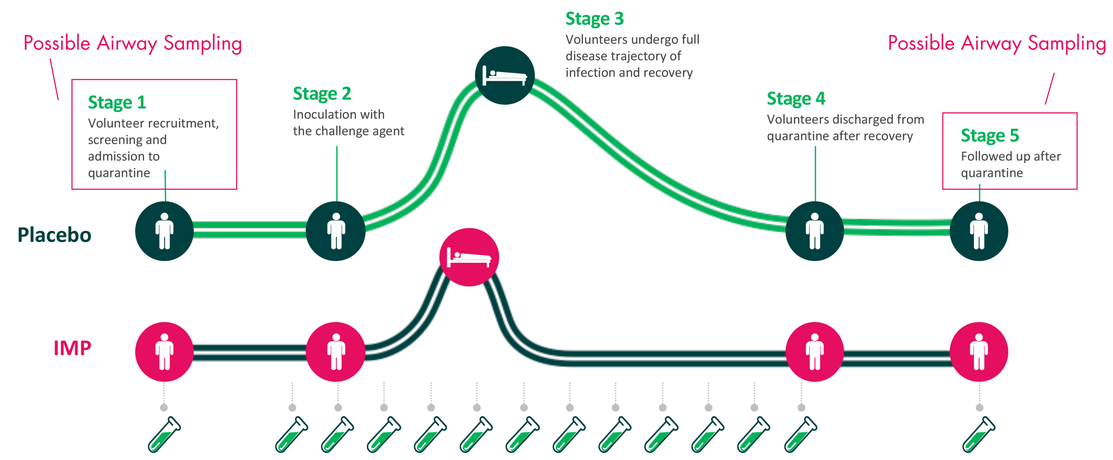
Figure 3: Typical experimental model of ALI infection presenting 2 different Multiplicity Of Infection (MOI), 1 containing drug and 1 Mock infection. Inoculum is left for 3 hours and then the apical surface is washed 3 times (approx. 10 minutes each).

ACKNOWLEDGEMENTS

The volunteers, our collaborators, hVIVO clinical, laboratory and operational teams.

hVIVO'S HUMAN CHALLENGE MODEL

Figure 4: Experimental model of human infection with respiratory viruses. Airway epithelium can be obtained either pre- (stage 1) or post human challenge core study (stage 5).



AIR–LIQUID INTERFACE RESULTS

CASE Study 1: H3N2 A/England/7762/2022

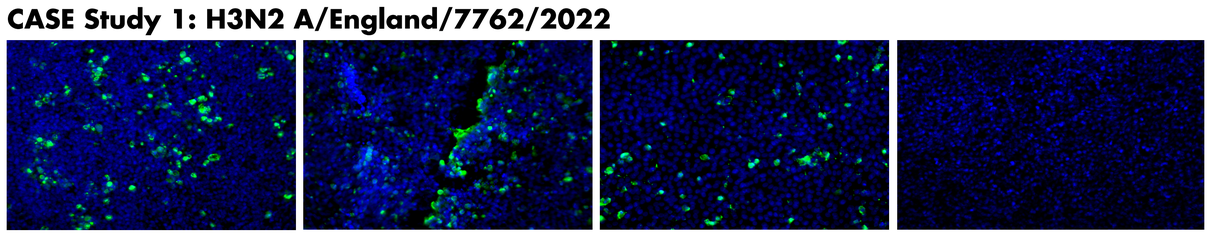


Figure 5: Immunofluorescence staining of ALI cultures infected with influenza A/England/7762/2022 at 24 hours (left), 48 hours (middle) and 72 hours (right). Mock infection is shown on the far-right picture.

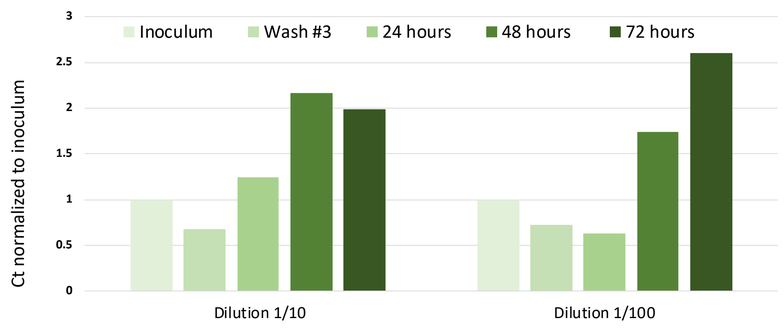


Figure 6: PCR of ALI daily washes of the apical surface cultures infected with influenza A/England/7762/2022. Ct values are normalised against the inoculum. 2 dilutions of inoculum were used (dilutions 1/10 and 1/100 of the neat stock). Neat stock titer is 6.71 Log_{10} $TCID_{50}$/mL on MDCK cells. Results are showing the kinetics of viral replication.

Increasing Ct normalised to inoculum represents the fold increase in Ct detected relative to the original inoculum. Immediate washing was used to remove residual non-attached virus. The subsequent increase in Ct over time demonstrates clear evidence of viral replication over time, peaking at 48 and 72 hours after viral inoculation for 1/10 and 1/100 virus stock dilutions respectively.

CASE Study 2: B/Connecticut/01/2021 (B-Victoria Lineage)

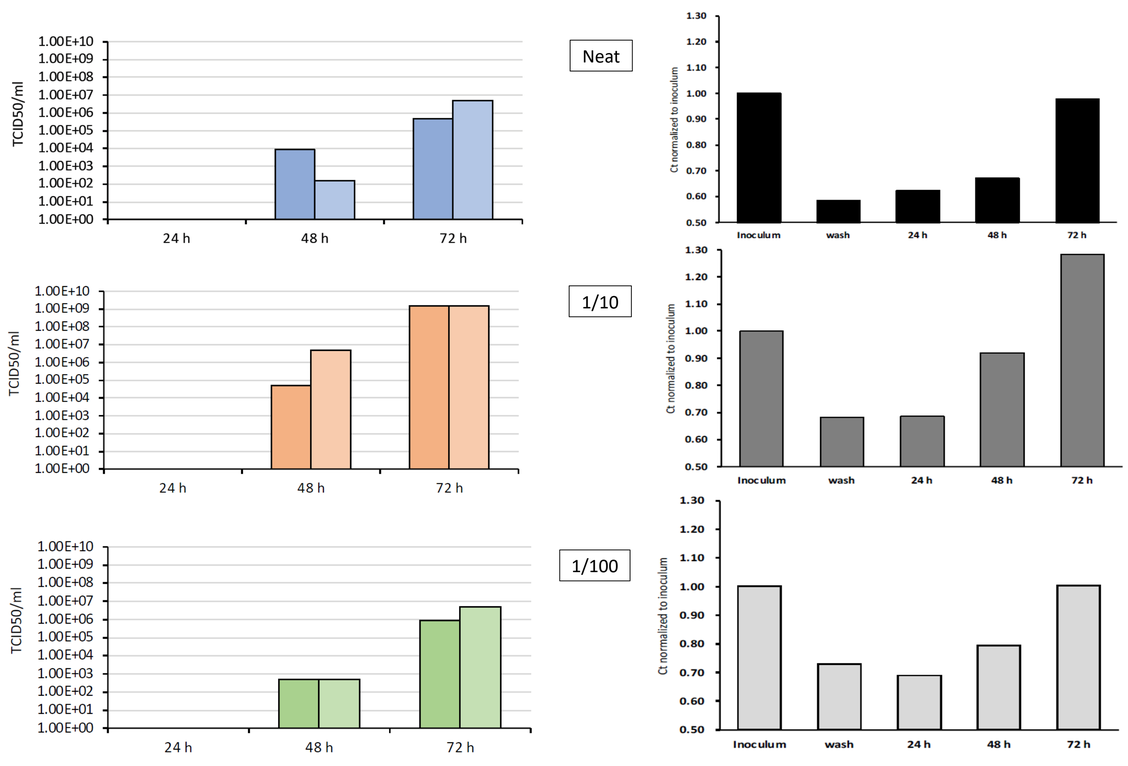


Figure 7: TCID50 (left panel) and PCR (right panel) of ALI daily washes of the apical surface cultures infected with influenza B/Connecticut/01/2021 (B-Victoria Lineage). Ct values are normalised against the inoculum. 3 dilutions of inoculum were used (Neat, 1/10 and 1/100). Neat Stock titer is 7.29 Log_{10} $TCID_{50}$/mL on MDCK cells.

After inoculation of the ALI cultures with B/Connecticut/01/2021, a wash was collected at each timepoint and analysed by qPCR and $TCID_{50}$ assays (Figure 7). The results show optimum results for virus replication were achieved after a tenfold dilution of the 7.29 log_{10} $TCID_{50}$/mL stock virus, with >10^9 log_{10} $TCID_{50}$/mL virus obtained by $TCID_{50}$ assay 72 hours after inoculation of the ALI cultures.

CONCLUSIONS

ALI cultures are an excellent tool to assess viral inoculum or drug impact. They provide the best possible in vitro solution as they are as close to the real natural situation as possible. Sampling from challenge study subjects will facilitate a direct comparison to ALI and human infection, providing powerful data.