Understanding SARS-CoV-2 transmission using experimental challenge

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Theme 5 lead

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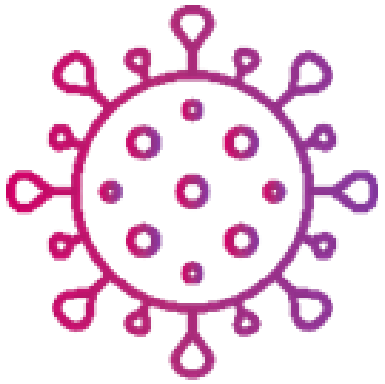

G2P-UK

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hVIVO

better treatments, faster

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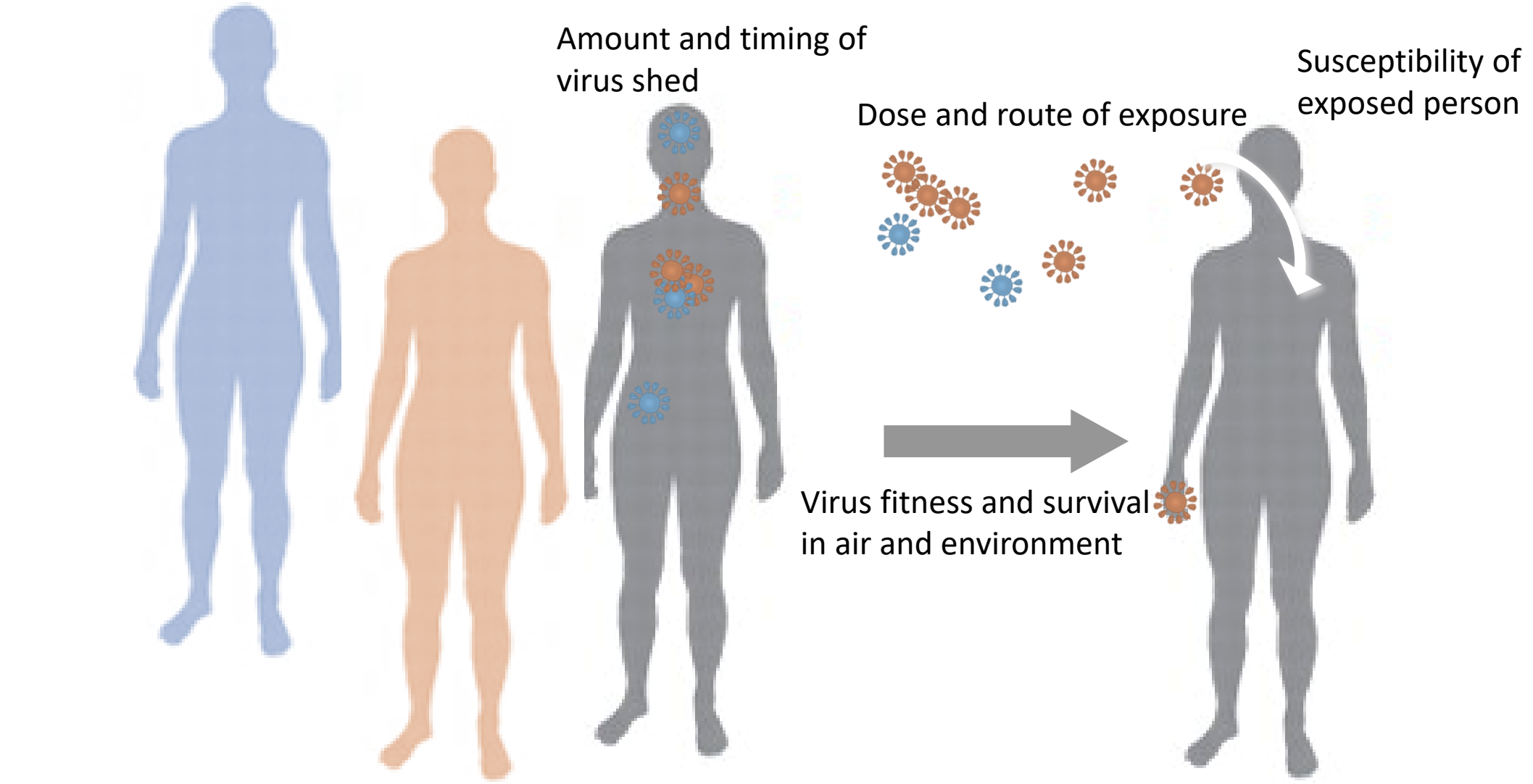
Respiratory virus transmission is complex and heterogeneous



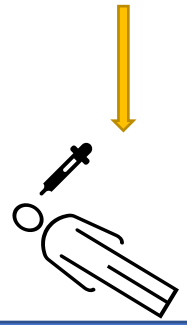
Experimental infections

- Controlled dose and strain of virus and route of infection
- Controlled environment and behaviour
- Naïve healthy young individuals

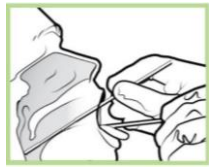
- Detect differences in virus shedding
- Study very early events after infection

Experimental Human Infection with SARS-CoV-2

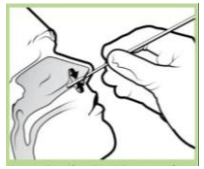
SARS-CoV-2 B.1.238
Intranasal inoculation
10 $TCID_{50}$ in 200ul



Throat swab



Mid-turbinate swab



Mask sampling

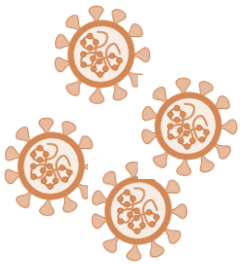
Air sample
taken by Coriolis
300 L/min x 10 min

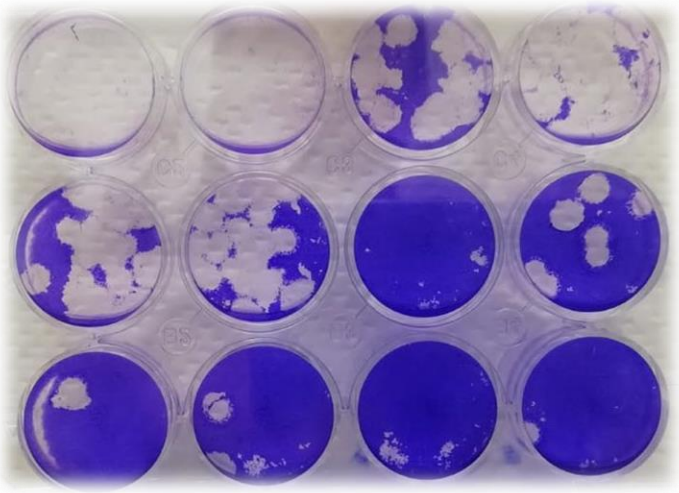
Day 0 1 2 3 ……. 14

Day 1 – Day 14
1) Throat and mid-turbinate swabs
2) Mask breath and air
3) Surfaces and hands

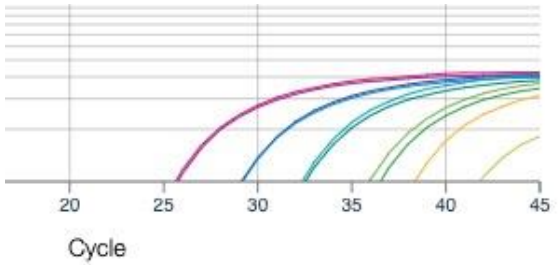
Viral load quantified by

Plaque assay – infectious viruses

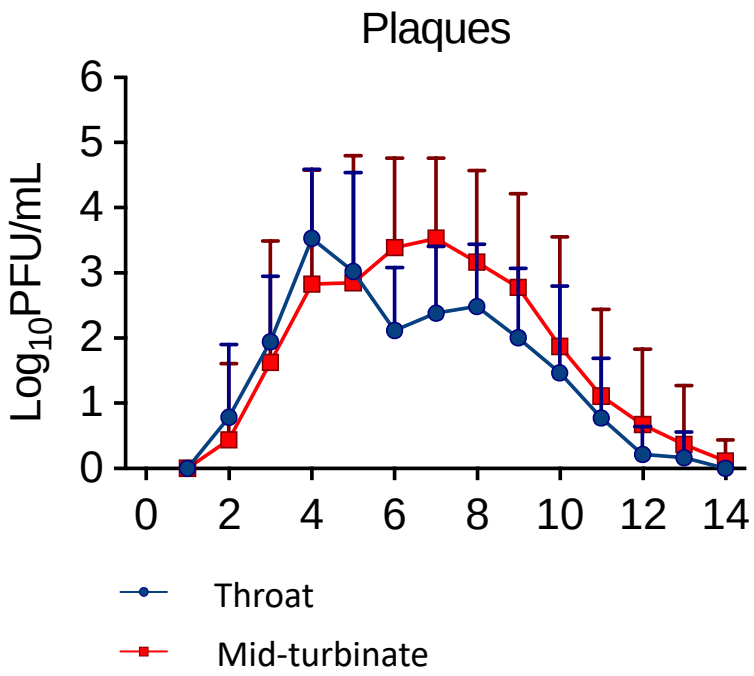


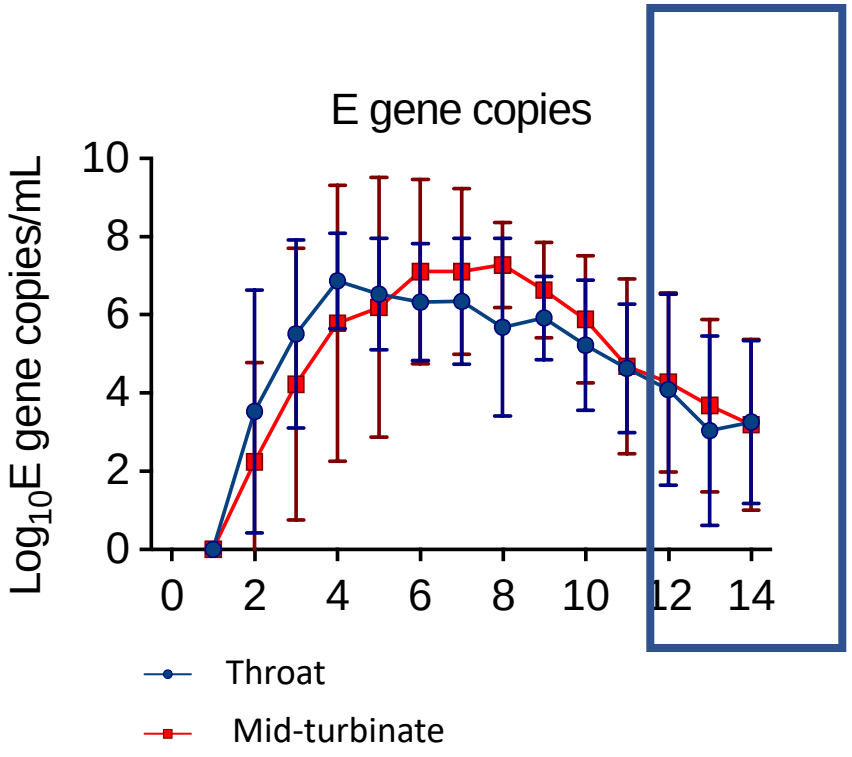


qPCR – Viral RNA - E gene

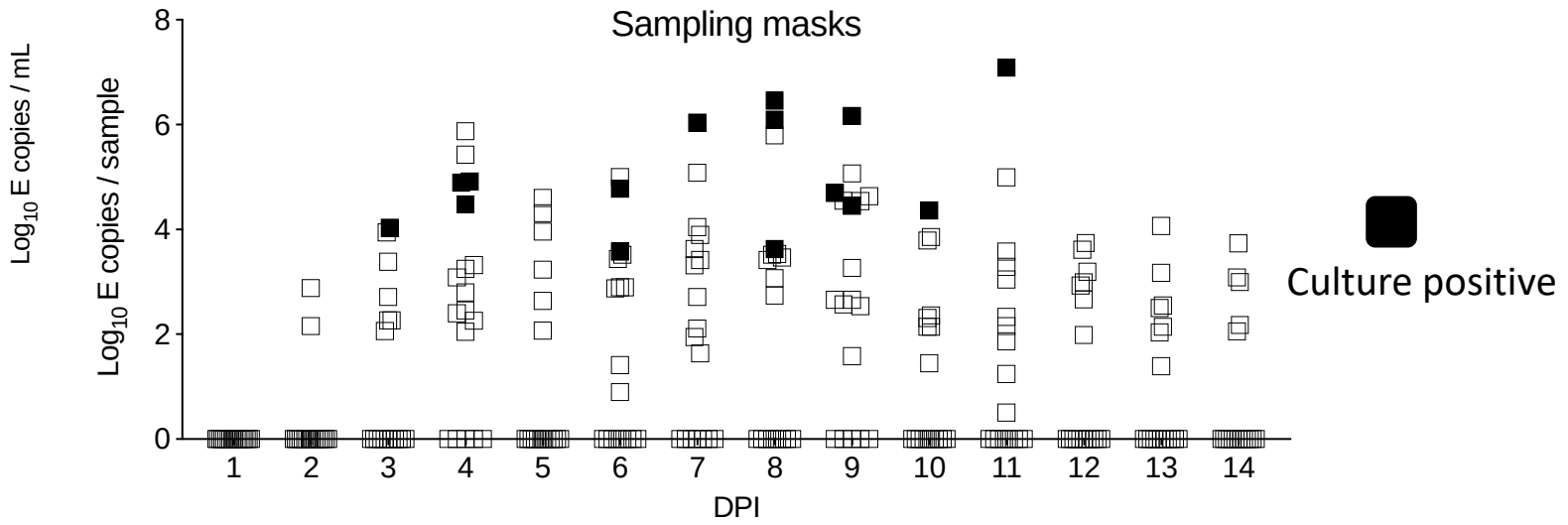


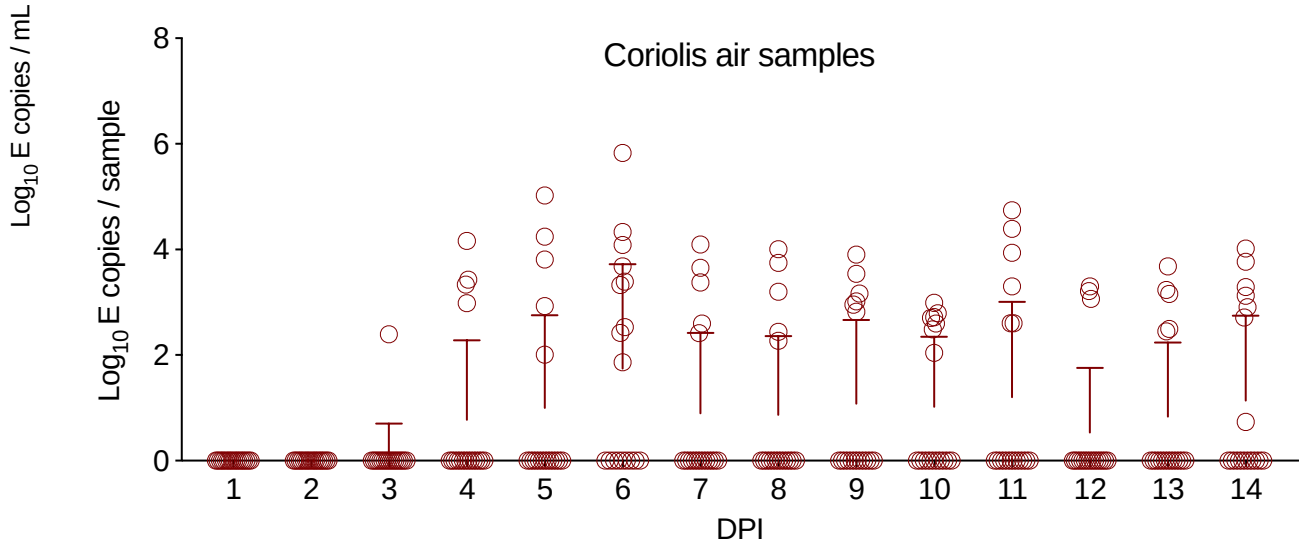
18 of 36 challenged healthy naïve volunteers became infected and uniformly shed high viral loads in throat and nose



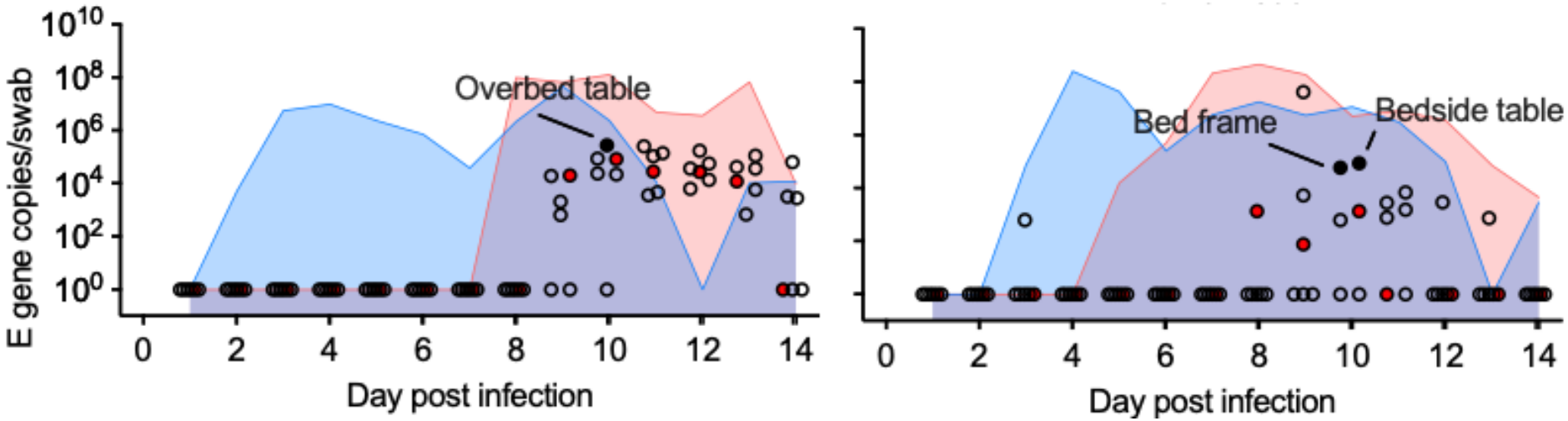


Virus was detected in mask and air emitted from infected volunteers



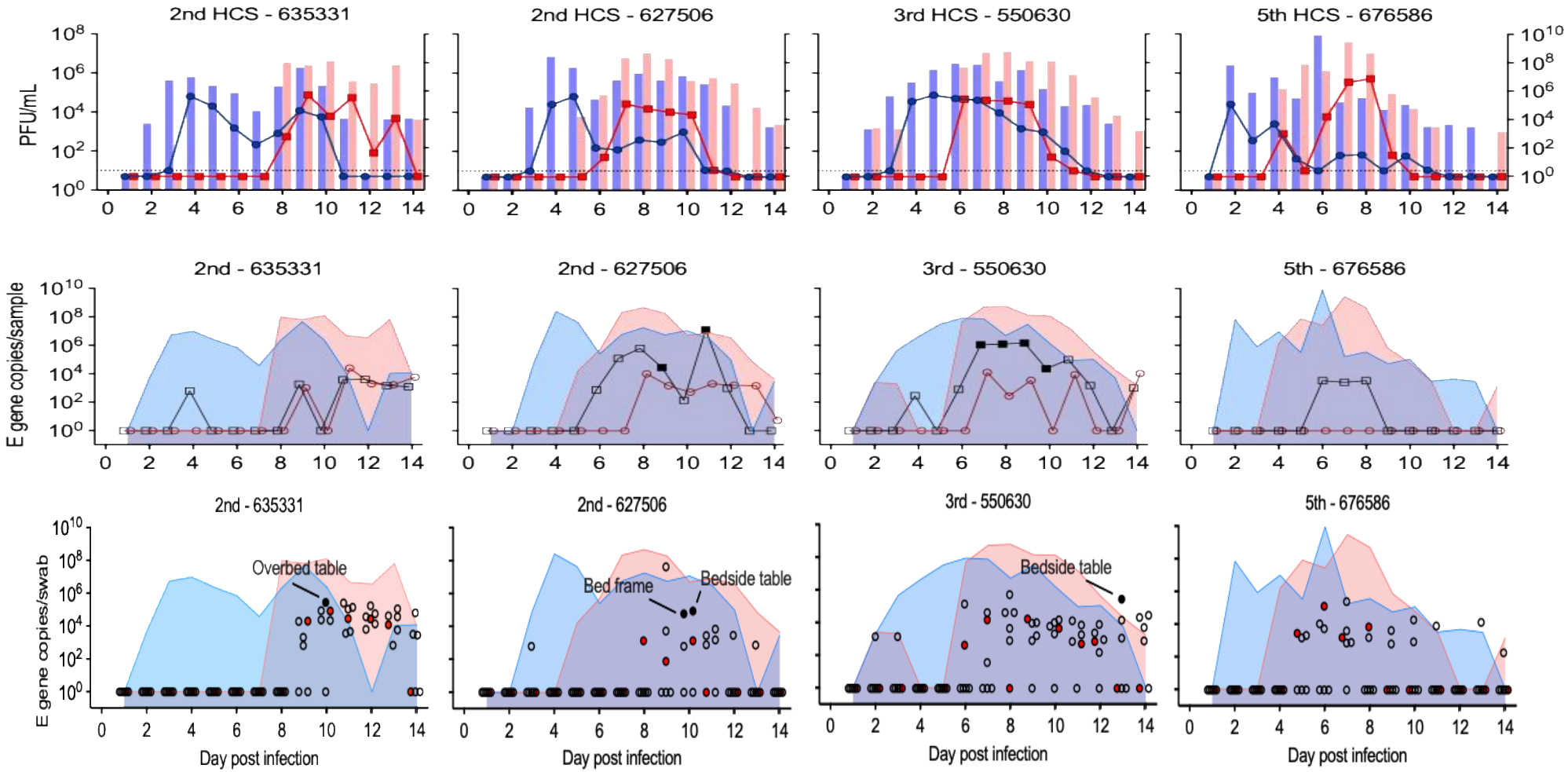


Hands and environmental surfaces were contaminated by infected individuals.



Viral load detected from hands (red circles) and environmental surfaces (open circles).
Solid black circles mean positive by viral culture

Virus was detected in mask and air, and environment was contaminated when nasal swabs were positive

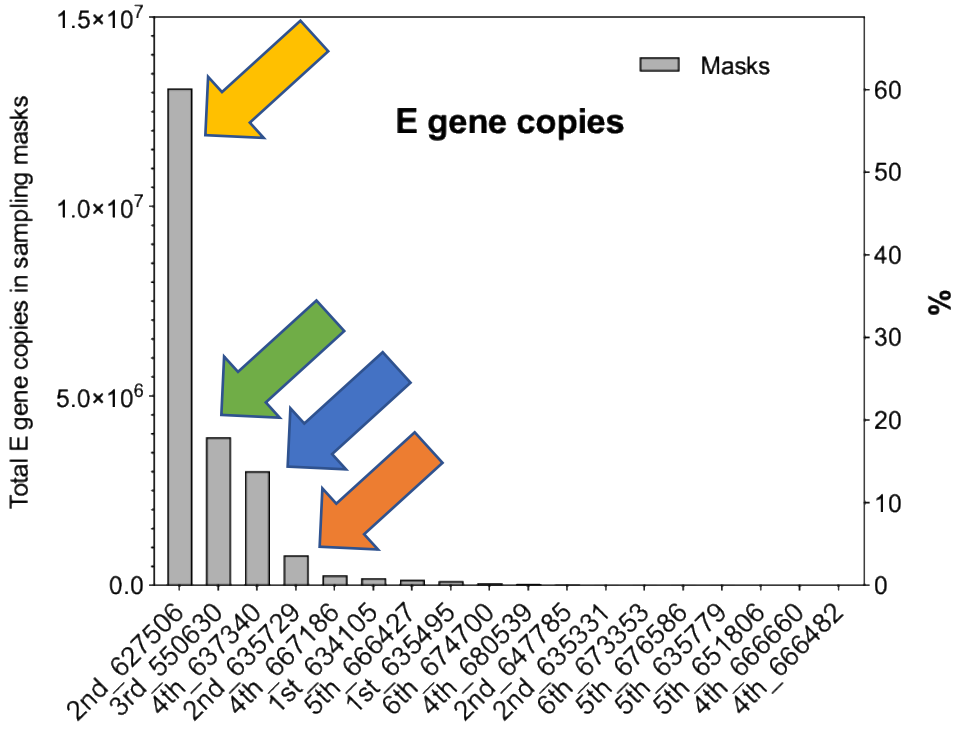


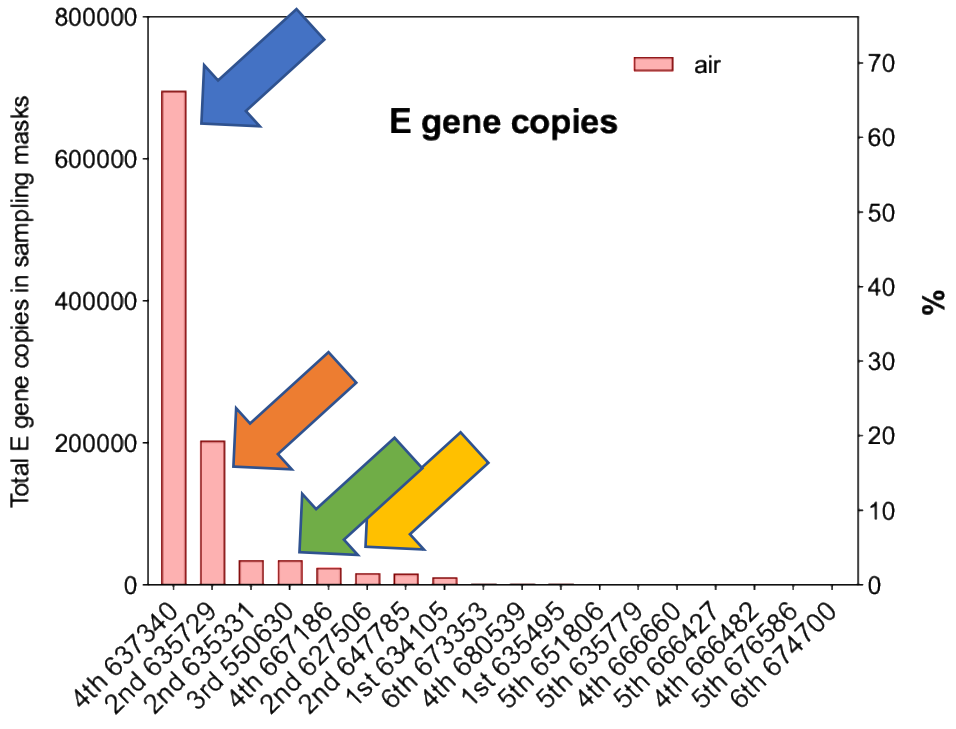
The correlation between nasal viral load in viral emissions

Nasal disinfectants could
temper the spread of SARS-CoV-2

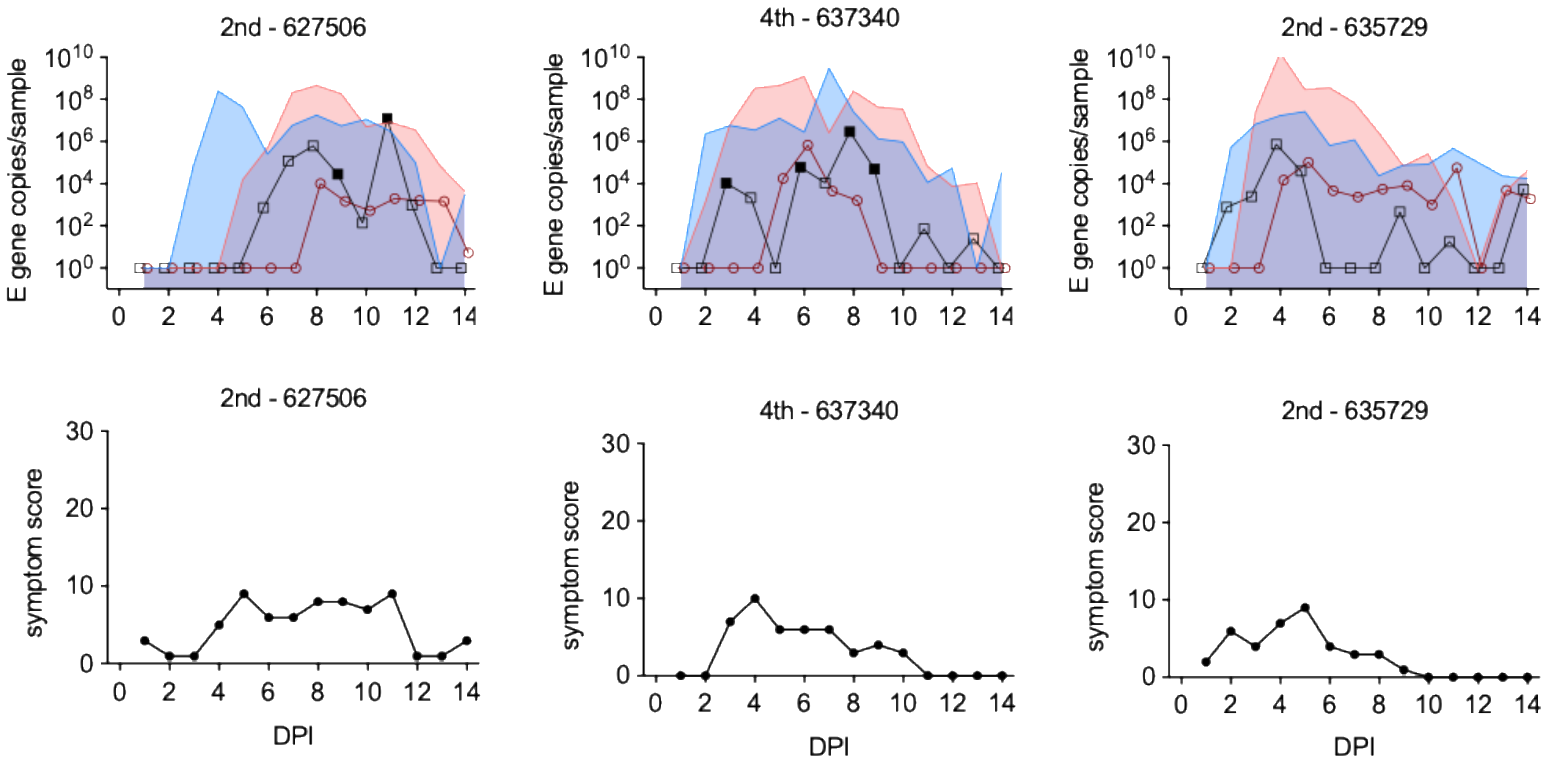
Covering the nose
is important

A few volunteers shed most of the virus: who are they?

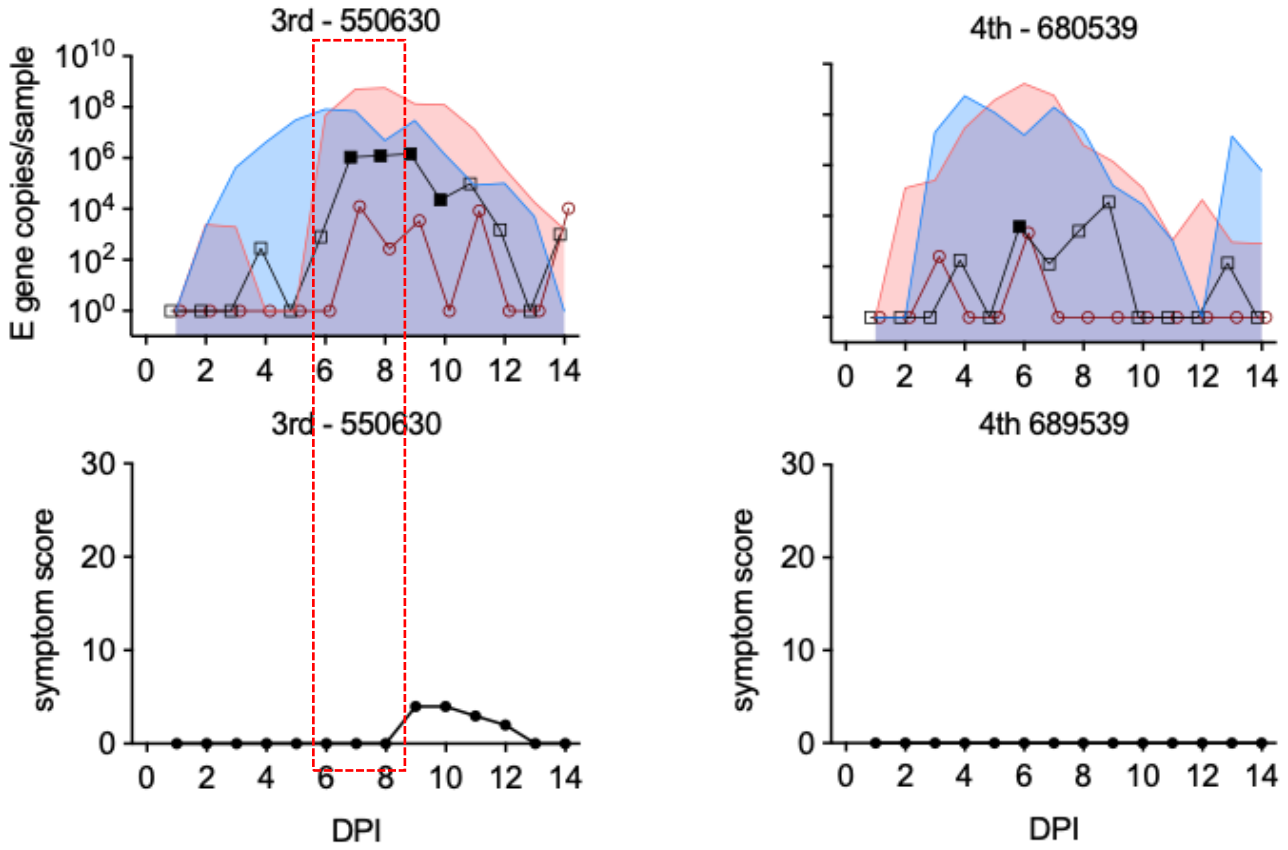




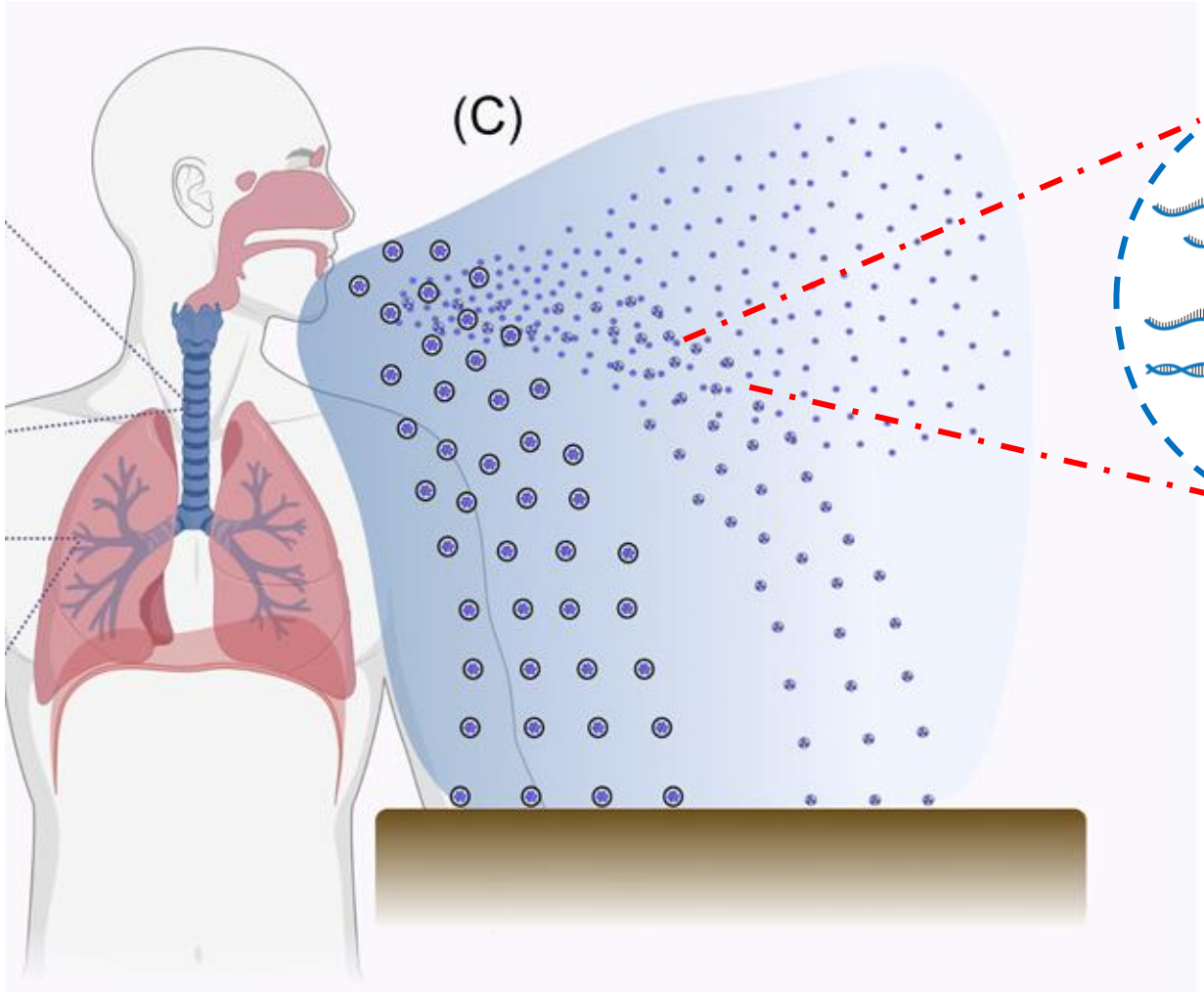
Virus emission sometimes coordinated with symptoms and nasal viral load



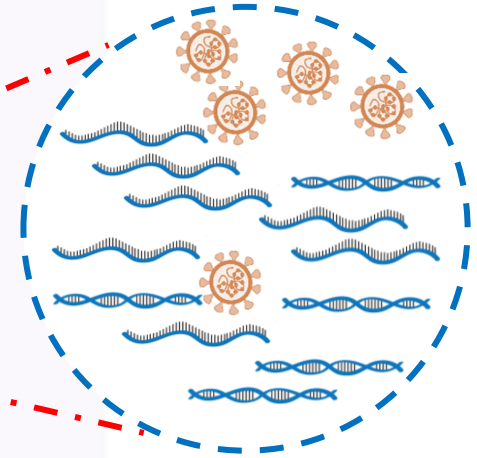
Virus emission can occur before or in absence of symptoms



Why are a minority of people highly infectious?

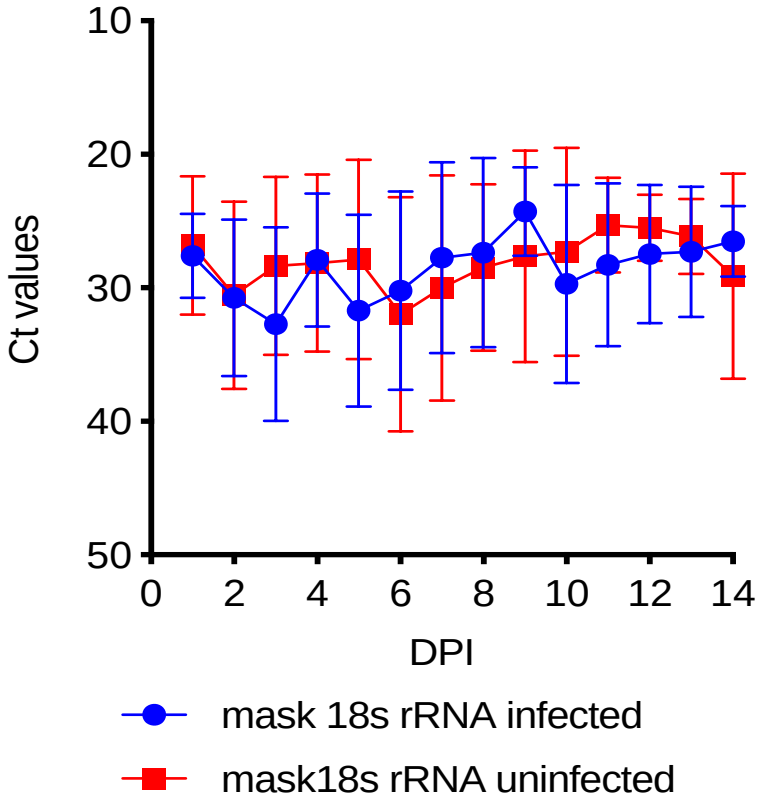


Viral RNA of SARS-CoV-2



Human housekeeping genes
RNaseP (Ribonuclease P)
18s rRNA (18S ribosomal RNA)

Some people emit more material than others…. Even if not infected!



There was no difference in internal controls between infected and non-infected volunteers

Internal controls did not increase during infection in infected volunteers

Infected volunteers who emit more virus also exhale more human material

What we have learned about transmission from human challenge study

1. Heterogeneity in transmission

- We detected virus in breath, air on hands and on environmental surfaces in rooms of infected volunteers.
- Despite equal virus dose and timing and route of infection, volunteers showed heterogeneity in quantity and timing of virus emitted.
- Volunteers who emitted most virus in air also emitted most human marker.
- This implies there are host determinants of infectiousness.

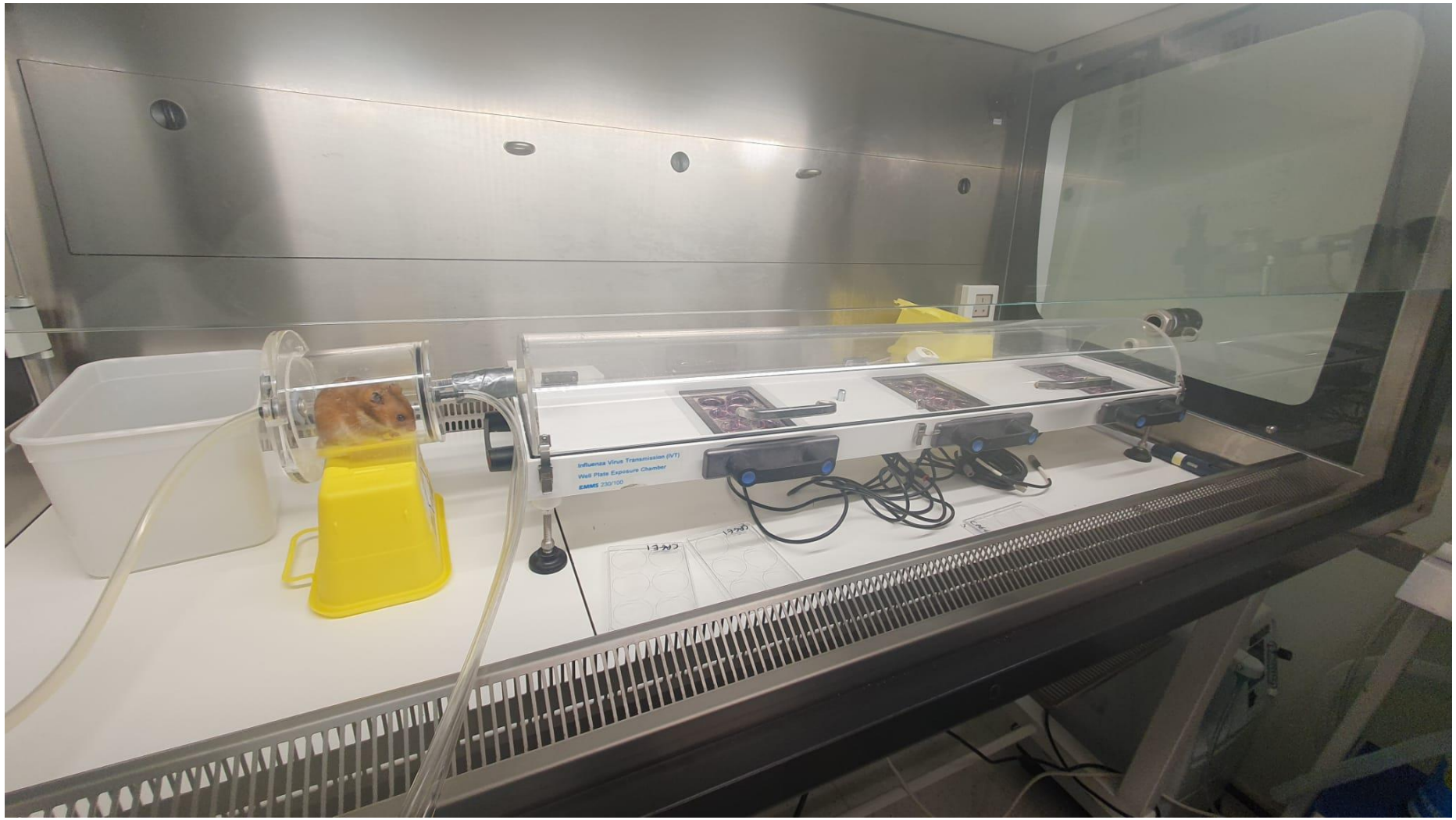
2. Timing of transmission

- Virus emission correlated with viral load in the nose.
- Most infected volunteers emitted virus coincident with symptoms.
- A few infected volunteers emitted virus to the air before any symptoms were recorded. Asymptomatic volunteers can emit virus to the air.

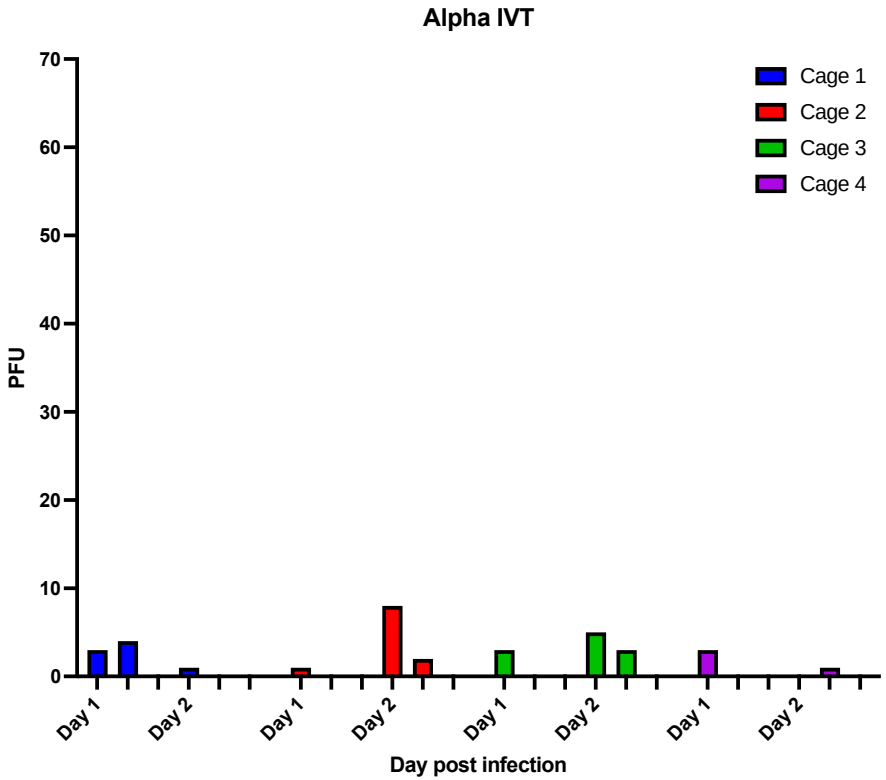
What next? Delta….

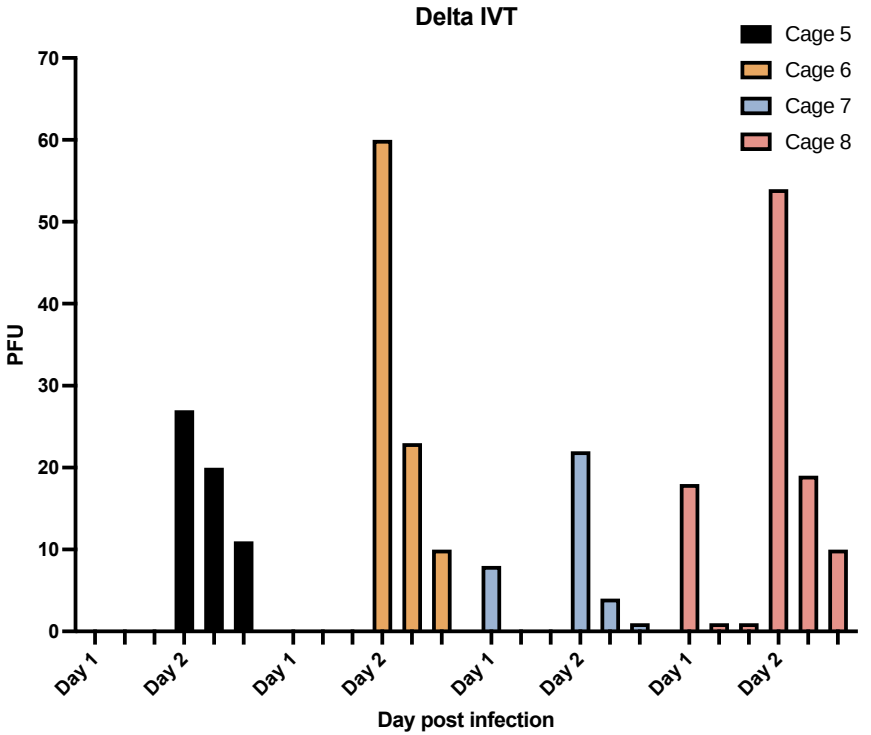
- Delta VOC has displaced other SARS-CoV-2 variants due to higher transmissibility
- Can we have a model that reflects higher transmissibility?
- Can we explain it mechanistically?

Measuring exhaled infectious virus from experimentally infected hamsters



Hamsters infected with delta VOC emit more infectious virus





Take home messages

- Experimental infections allow us to understand host factors and viral factors that determine SARS-CoV-2 transmission
- A minority of individuals emit the majority of infectious virus
- Some virus variants are emitted more efficiently

- A delta challenge virus is being manufactured for human challenge studies, in vaccinated volunteers

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Isolator for **M**easuring **A**erosol and **D**roplet **GEN**eratio**N**

Nicola Yaxley and Susan Gould

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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IMADGENN

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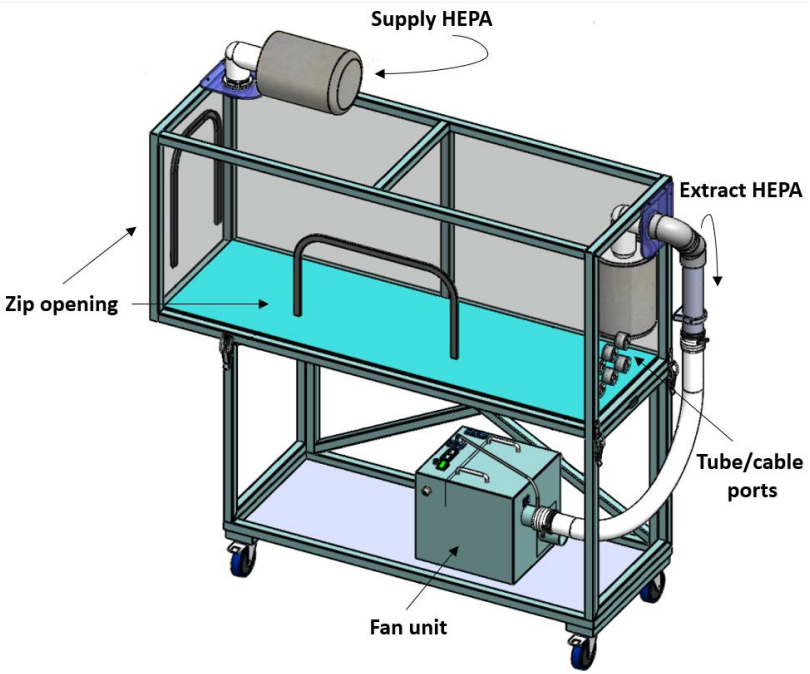
- Healthy volunteers are asked to perform various respiratory exercises directed into the flexible film isolator
- Air samplers and settle plates used to sample respiratory aerosols and droplets
- Using respiratory bacteria as a marker for respiratory pathogens
- Counts of airborne *Streptococcus salivarius* have been shown to be significantly related to the risk of classroom transfer of measles (Reid et al. 1956)

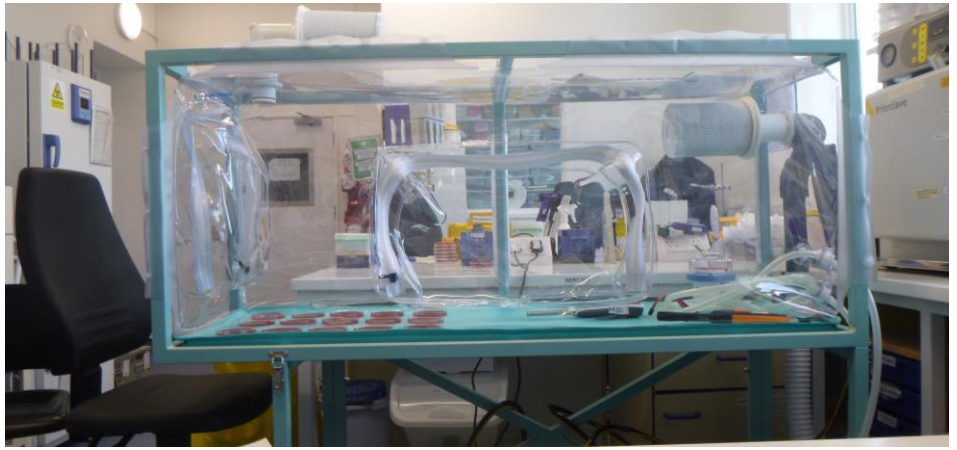
So far…

- IMADGENN has been used to test the efficacy of face coverings

Work is in progress to…

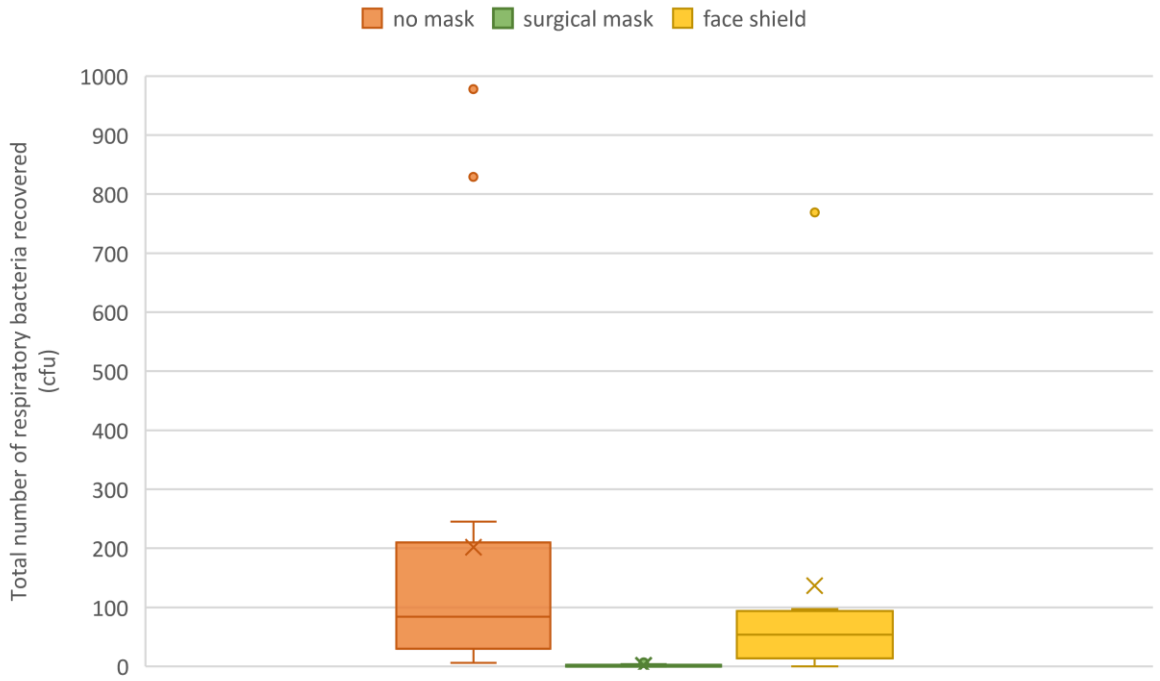
- Understand the risk of different respiratory behaviours
- Determine the size distribution of respiratory aerosol droplets and the bacteria they contain using the May cascade impactor





Face coverings

- Total respiratory/oral bacterial counts after volunteers had performed a sequence of respiratory exercises (deep breath through nose and mouth, coughing, counting, and singing)



- Face coverings are effective in reducing droplet and aerosol dispersal
- The level of effectiveness can be affected by factors such as gender, and if male, the presence of facial hair

Cascade Impactor

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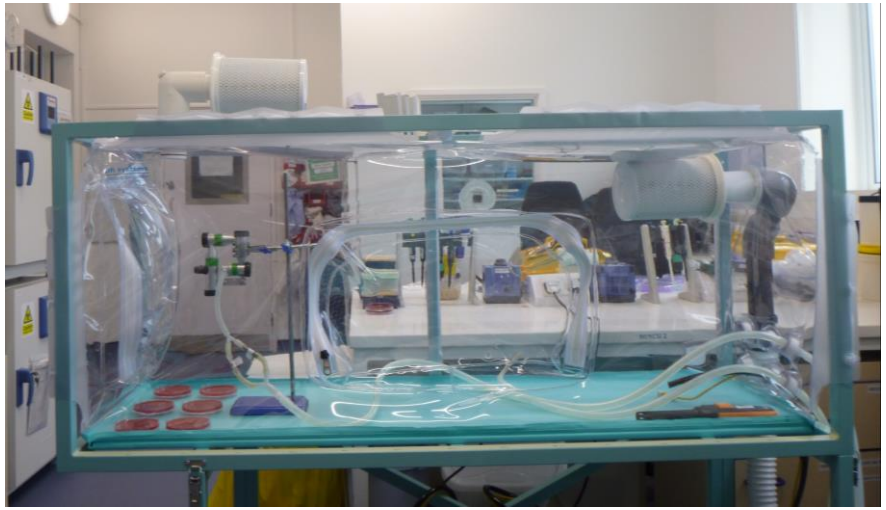
- Using the May cascade impactor we aim to determine the size distribution of respiratory aerosols
- Four progressively smaller jets direct aerosolised samples onto coated glass slides
- Samples with a size distribution between 50 – 1.5 µ show greatest efficiency (overall sample size range >1 µ and ≤ 200 µ)



Participant	Total respiratory/oral cfu				
	Slide 1 >20 µ	Slide 2 <20 µ	Slide 3 <7 µ	Slide 4 <3 µ	10/20 cm centre settle plate
1	~140	2	2	1	320/310
2	0	2	0	0	-
3	0	1	2	1	-
4	2	9	2	0	105/55
5	0	3	2	0	228/59

Very early results!!

Distribution of Droplet Dispersal

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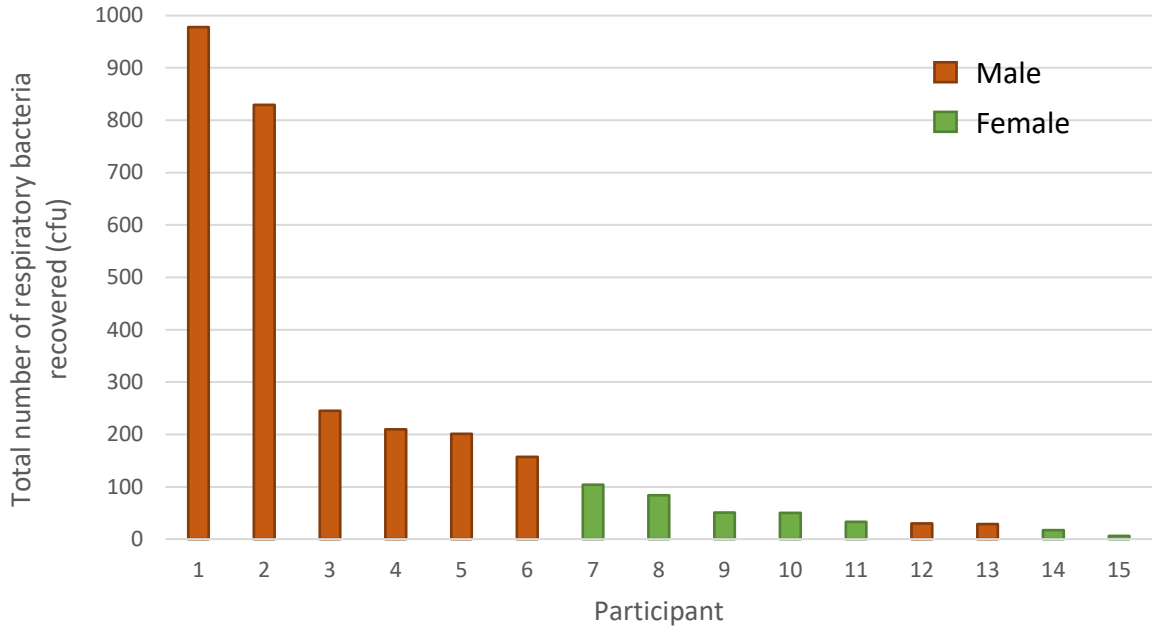
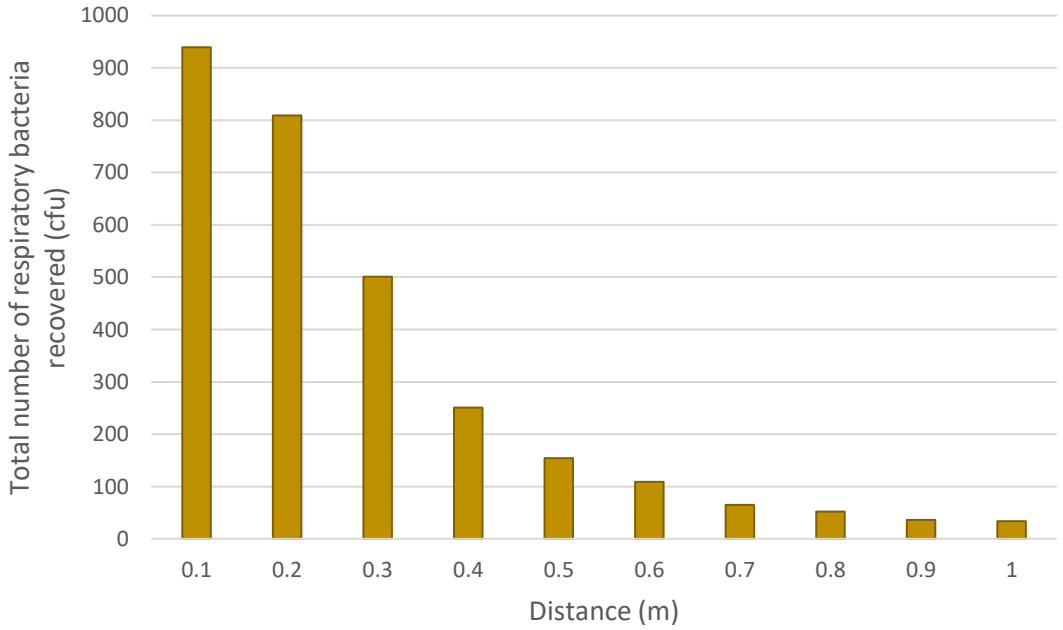
- Total respiratory/oral bacterial counts after non-mask wearing volunteers had performed a sequence of respiratory exercises (deep breath through nose and mouth, coughing, counting, and singing)





IMADGENN in Liverpool

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Participants:

- Early-stage infection (<5 days)
- Community cases

Location:

- Clinical Research Facility, Royal Liverpool University Hospital

Sample Collection:

- Series of respiratory manouevres
- 10 minutes sampling

Sampling

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IMADGENN - samples
- Near patient
- Sartorius style filter
- Anderson sampler

Additional sampling:
- Wearable button sampler
- Room/household air sampling using Sartorius MD8 Airport sampler

Gelatin filters
- Dissolved in DMEM
- PCR
- Culture

Participants

- 25 adults recruited July-November 2021
- 21 used all 3 types of sampler including IMADGENN
- 11 male, 10 female
- Median age: 31.5 years
- 1-7 days post symptom onset (median 4)

COVID-19 vaccinations	Participants
2	16
1	3
0	2

Preliminary results from 8 participants

	Room	Button	Near patient	Filter	Anderson stage					
					1 (>7um)	2 (>4.7)	3 (>3.3)	4 (>2.1)	5 (>1.1)	6 (>0.65)
1										
2										
3										
4										
5										
6										
7										
8										

- Targets = N gene, Orf 1ab, S gene
- CT values mid-low 30s

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Thank you

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Dr Ginny Moore, Patricia Barkoci, Wilhemina D'Costa, Simon Parks, Allan Bennett Biosafety, UKHSA
Susan Gould
Tom Fletcher, Paul Garner, Lance Turtle, Allan Bennett, Jake Dunning

Thanks to all the volunteers!

sites.manchester.ac.uk/covid19-national-project

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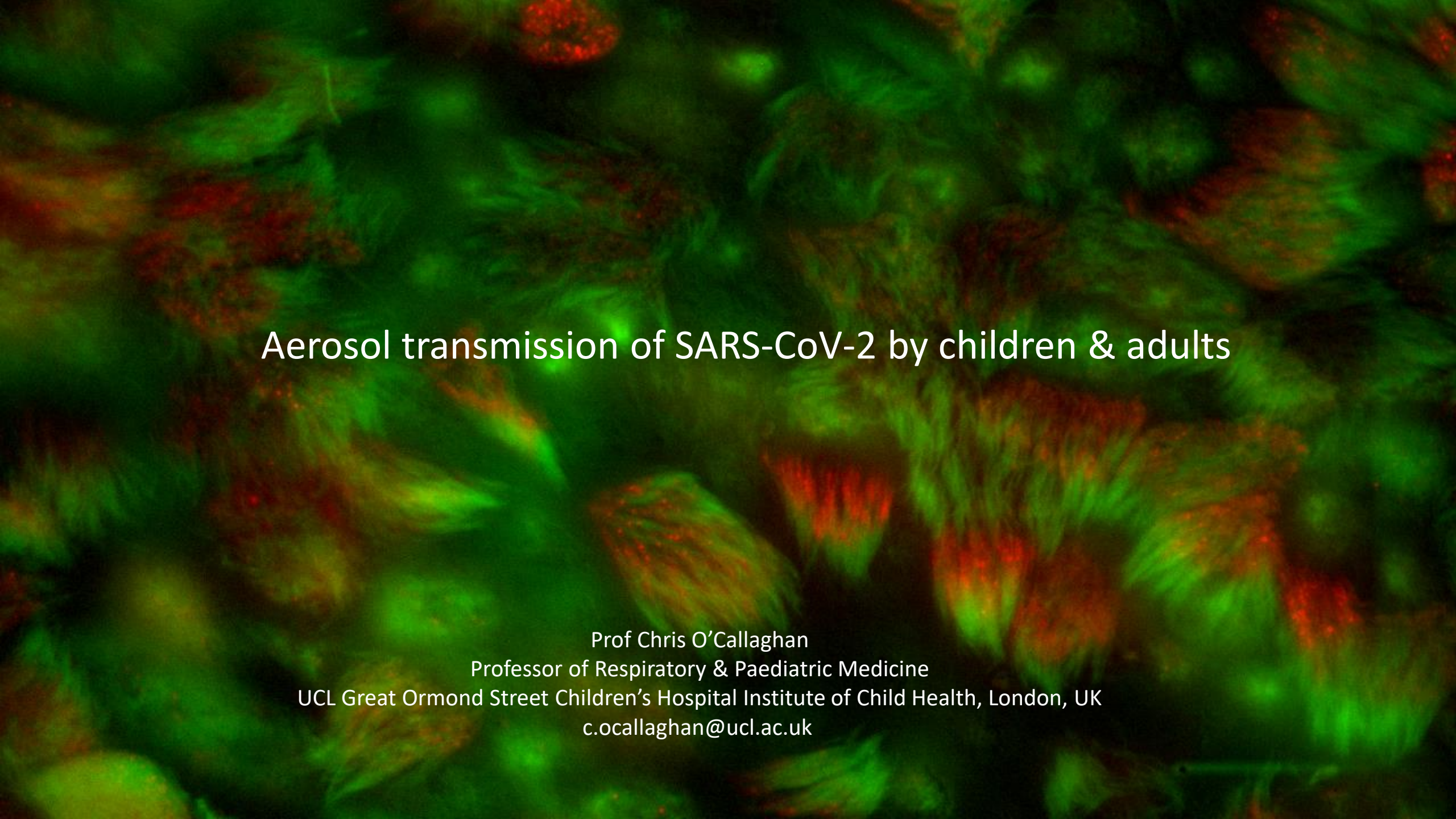
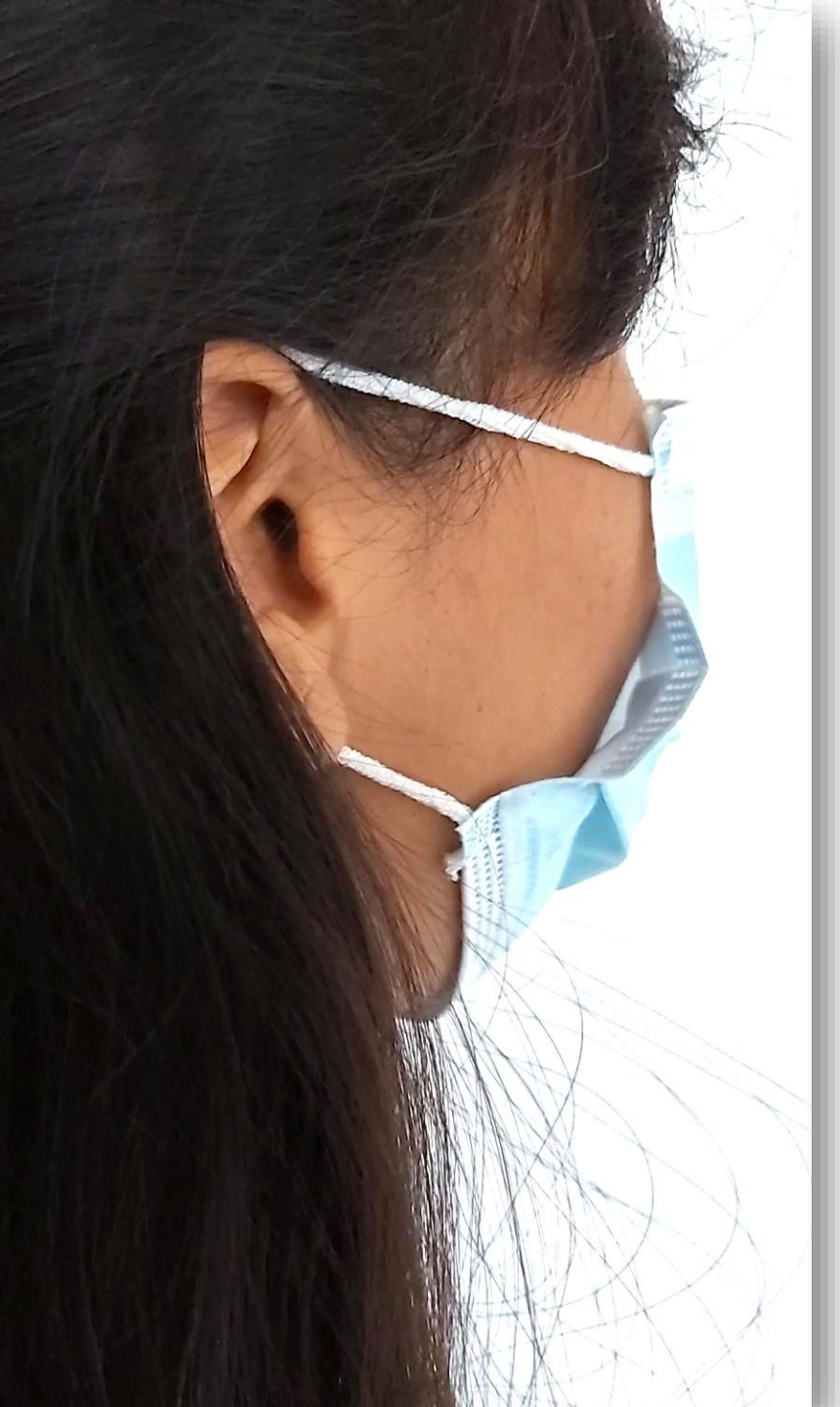
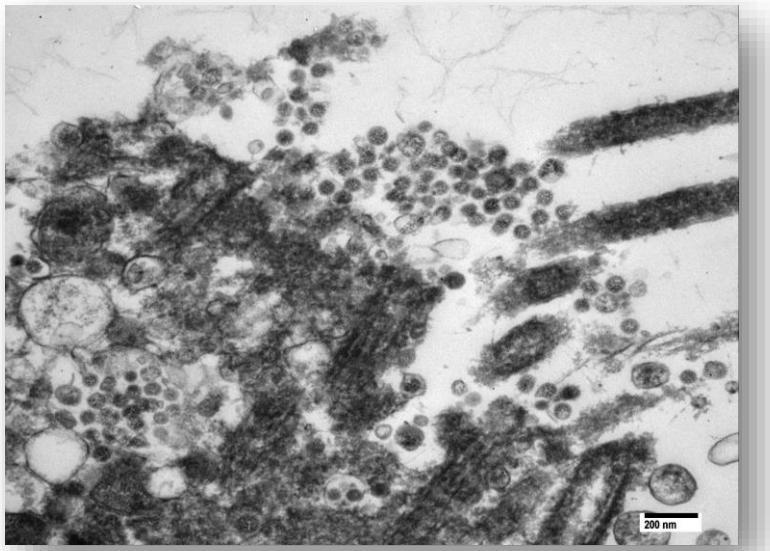


Aerosol transmission of SARS-CoV-2 by children & adults

Prof Chris O'Callaghan
Professor of Respiratory & Paediatric Medicine
UCL Great Ormond Street Children's Hospital Institute of Child Health, London, UK
c.ocallaghan@ucl.ac.uk



Respiratory viruses enter the body via the nose, mouth or eyes & infect cells

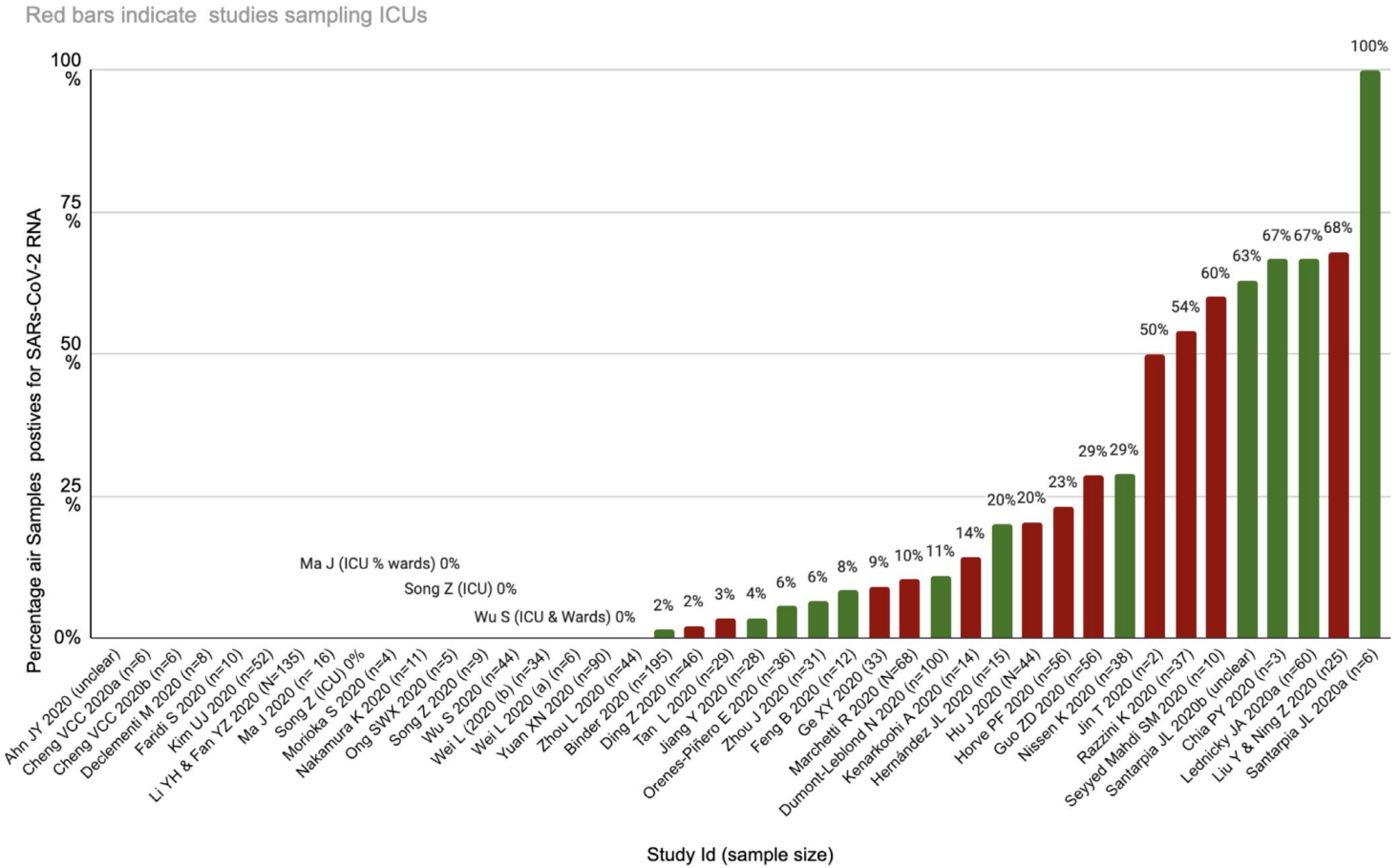


Preventing a respiratory virus from entering the nose, mouth or depositing in the eyes prevents infection

Early in a pandemic, lack of evidence, does not mean it does not occur

Did the interpretation of ‘lack of evidence’ slow the response to COVID-19 transmission?

Respiratory viruses leave the body via the nose & the mouth as secretions or large & small aerosol particles



The detection of SARS-CoV-2 RNA in the air cannot presume transmission, since only viable virions can cause disease.

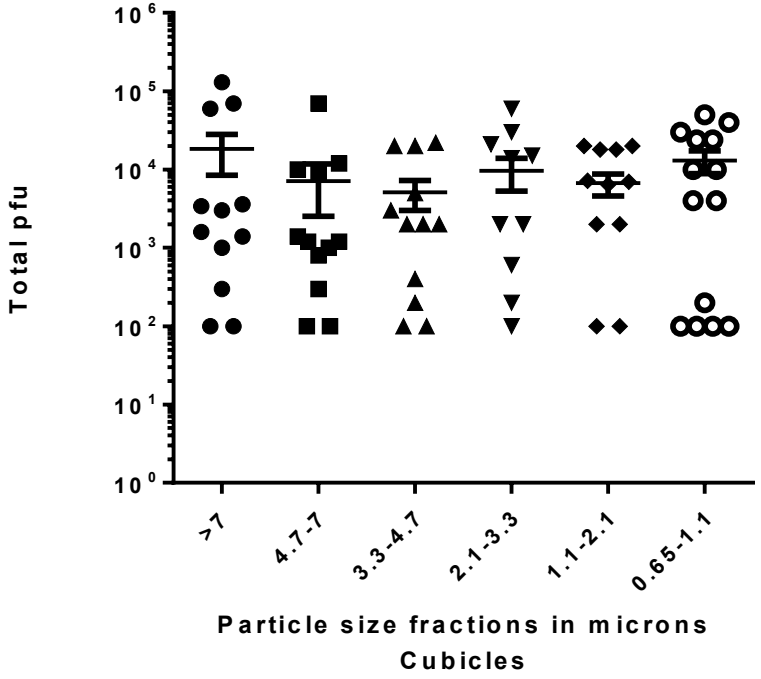
No airborne study to date definitively demonstrates SARS-CoV-2 is of an infectious nature, which offers the most robust evidence of transmissibility.

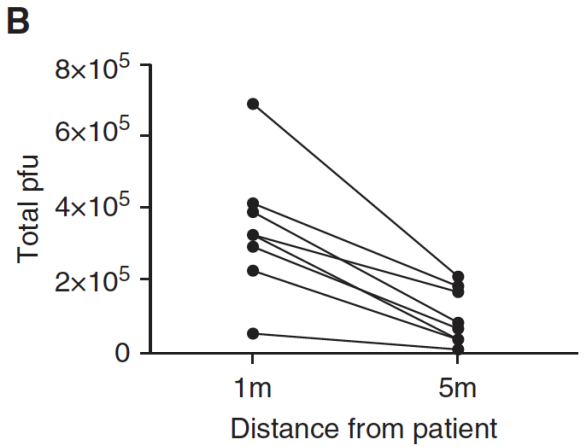
Heneghan C. F1000 Research

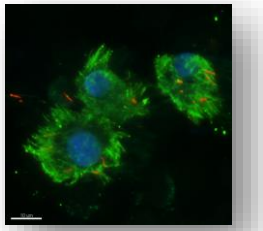
Evidence of Respiratory Syncytial Virus Spread by Aerosol
Time to Revisit Infection Control Strategies?

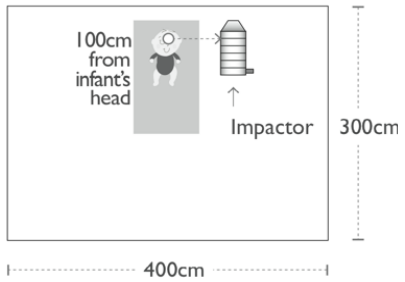
Hemant Kulkarni[1], Claire Mary Smith[2], Dani Do Hyang Lee[2], Robert Anthony Hirst[1], Andrew J. Easton[3], and Chris O'Callaghan[2]

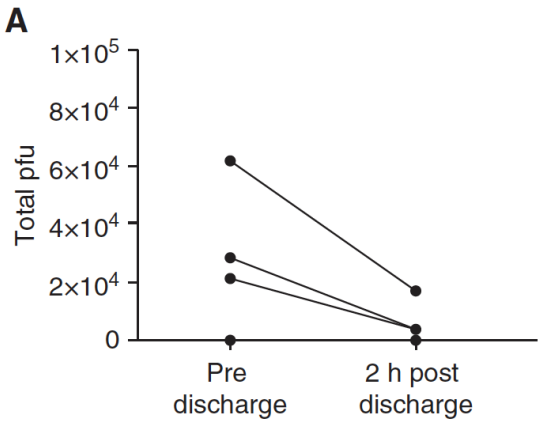
Kulkhani et al, American Journal of Respiratory & Critical Care Medicine; 2016: 194: 308-16

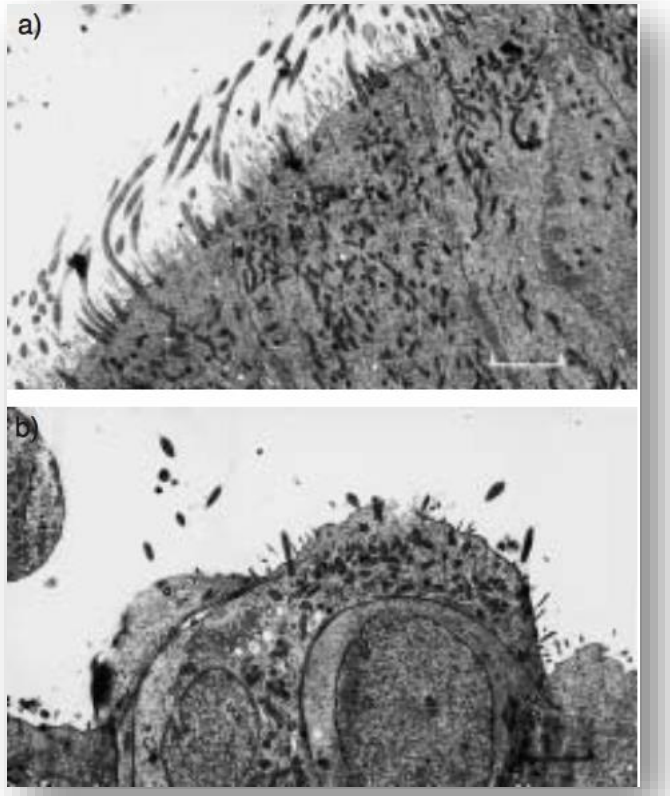








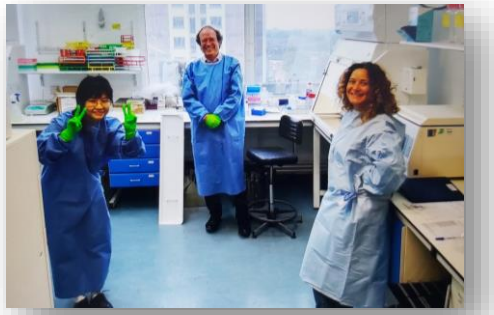




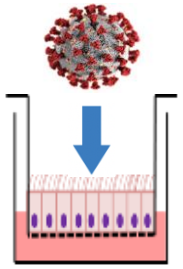
The effects of coronavirus on human nasal ciliated respiratory epithelium

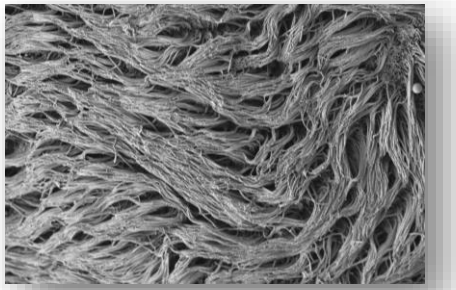
M.A. Chilvers*, M. McKean*, A. Rutman*, B.S. Myint#, M. Silverman*, C. O'Callaghan*

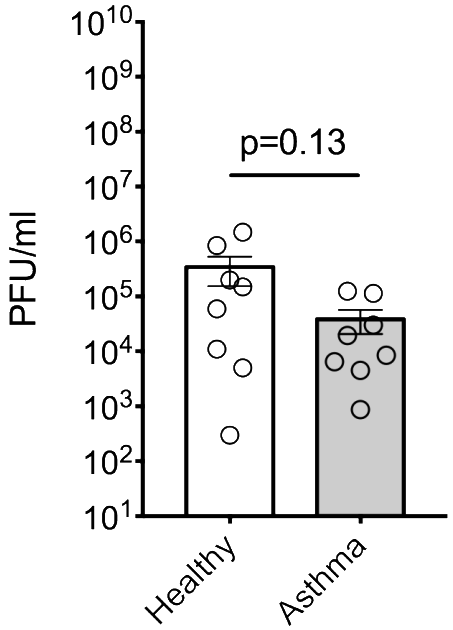
European Respiratory Journal: 2001:18: 965-70

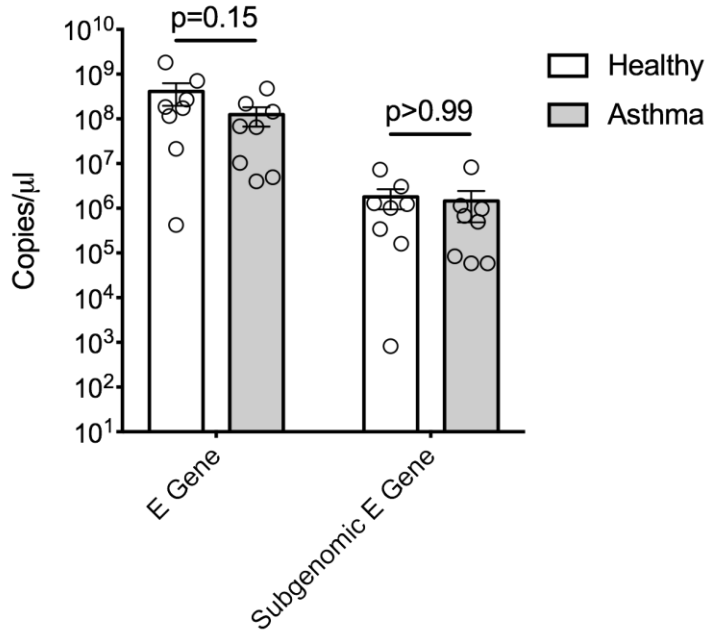


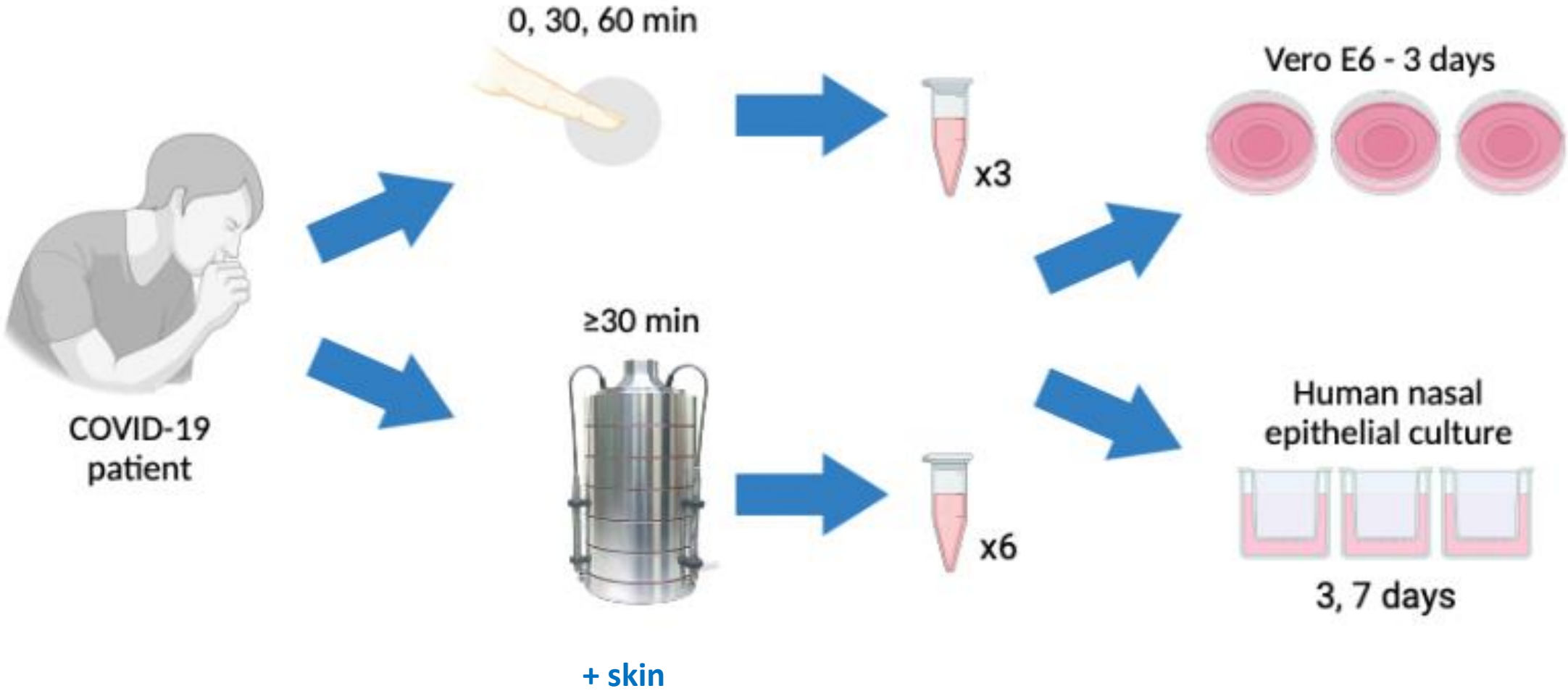
CL3 laboratory – Royal Free





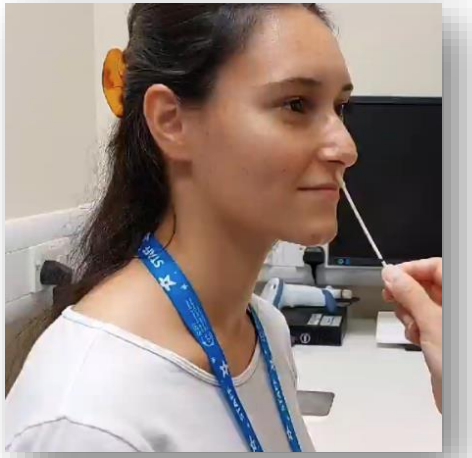


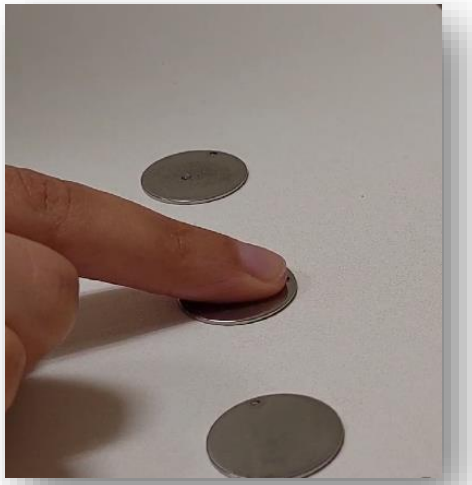




+ skin

- Unvaccinated and vaccinated children
- Aerosol generating procedures in children
- Vaccinated and unvaccinated adults
- Asymptomatic adults





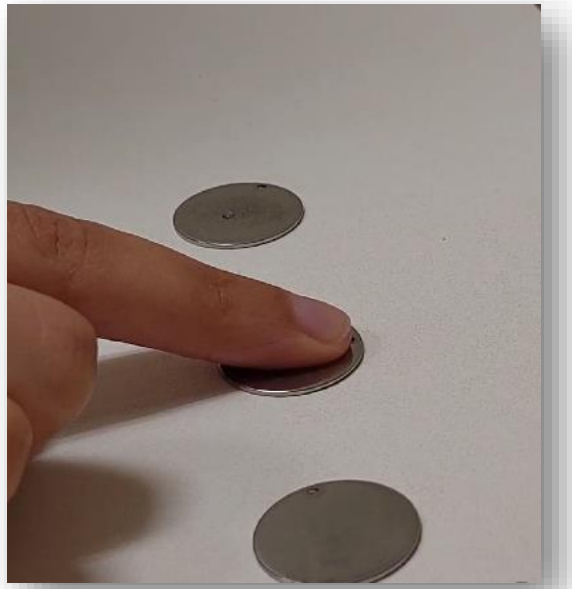
Adult double vaccinated COVID-19 +ve patients
Skin & Fomite (Cycle threshold - CT)

Day of illness	Nasal/throat swab	Skin swab	Disc 1	Disc 2	Disc 3
2	29.646	ND	X	X	X
1	17.169	33.614	19.954	22.005	X
3	21.417	ND	28.682	32.837	X
7	29.496	35.401	35.915	34.643	37.052
7	18.058	37.42	23.91	27.192	31.444
7	33.178	35.318	35.564	ND	40.602
7	25.34	35.75	31.057	32.687	31.45

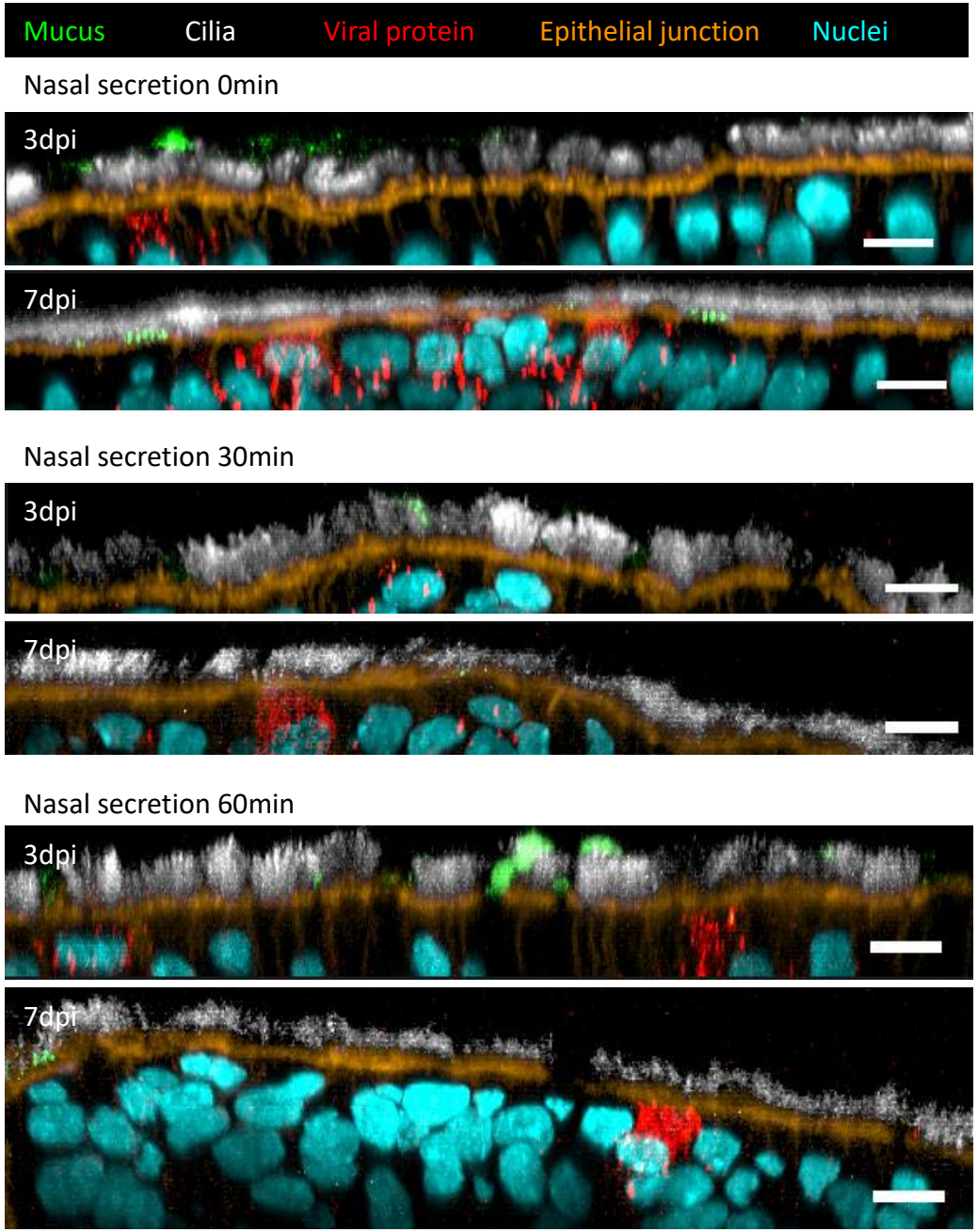
Low CT number suggests more virus

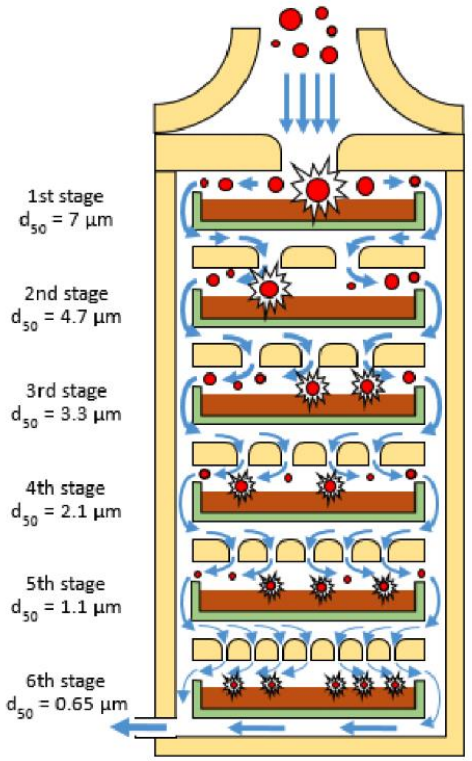
If viral RNA is found it does not mean there is infectious virus

Contact from finger to surface



Preliminary evidence that SARS-CoV-2 from those infected remains infectious for at least one hour





Adult double vaccinated COVID-19 +ve patients
Aerosol collection (Cycle threshold - CT)

Day of illness	Nasal/throat swab	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Filter	Funnel
2	29.646	ND	ND	ND	ND	ND	ND	ND	ND
1	17.169	ND	ND	ND	ND	ND	ND	ND	ND
3	21.417	ND	ND	ND	ND	ND	ND	ND	ND
7	29.496	ND	ND	ND	ND	ND	ND	ND	ND
7	18.058	ND	ND	39.562	ND	40.667	ND	ND	ND
7	33.178	ND	ND	ND	ND	ND	ND	ND	ND
7	25.34	ND	ND	ND	ND	ND	ND	ND	36.027

Low CT number suggests more virus

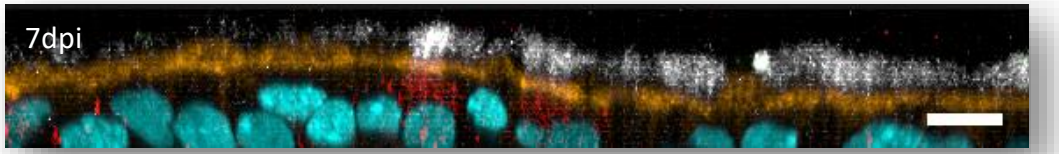
If viral RNA is found it does not mean there is infectious virus

Does a negative result mean there is no infectious virus present?

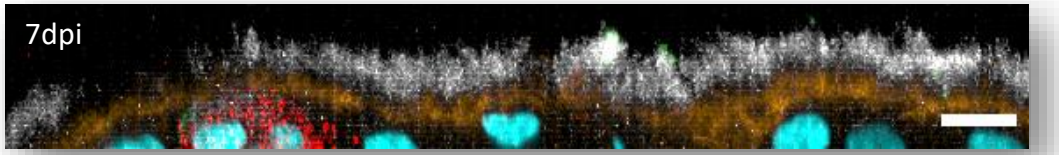
Addition of impactor samples to ciliated cultures

Mucus Cilia Viral protein Epithelial junction Nuclei

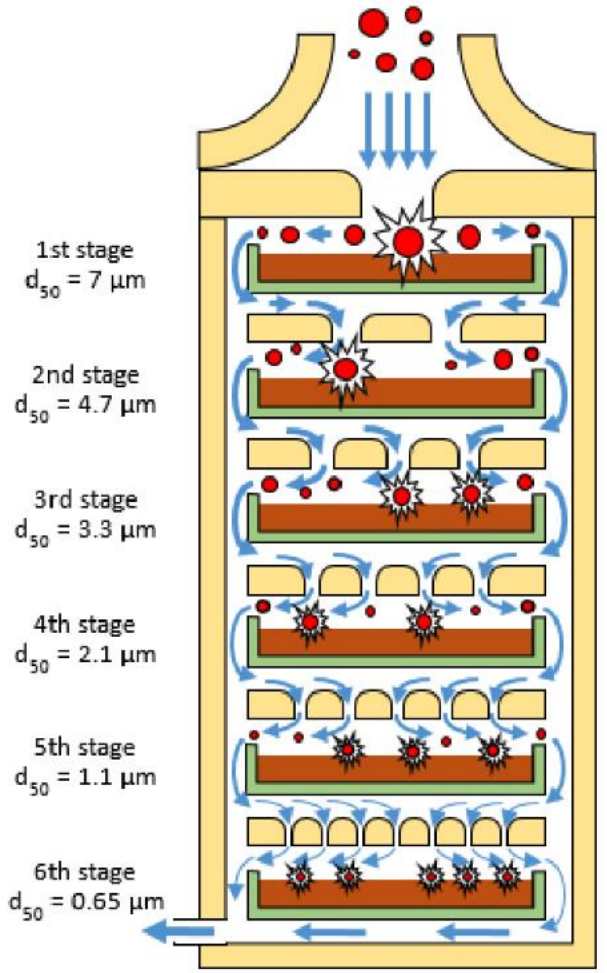
Impactor stage 03



Impactor stage 05



Preliminary evidence of SARS-CoV-2 infection of human ciliated epithelium



Each impactor stage collects aerosol of a different aerodynamic diameter

Does a negative CT mean there is no infectious virus present?

Children home visits (Cycle threshold - CT)

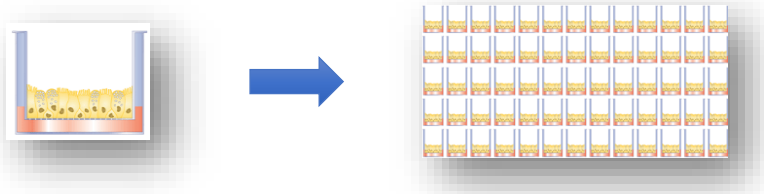
Day of illness	Nasal/throat swab	Skin swab	Mobile	Door handle	Disc 1	Disc 2	Disc 3
5	24.585	36.631	ND	39.791	35.528	34.724	33.168
3	36.571	36.014	ND	37.971	ND	ND	ND
4	24.197	36.234	ND	ND	34.668	36.027	38.559

Day of illness	Nasal/throat swab	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Filter	Funnel
5	24.585	ND	ND	ND	ND	ND	ND	ND	ND
3	36.571	ND	ND	ND	ND	ND	ND	ND	ND
4	24.197	ND	ND	ND	ND	ND	ND	ND	33.973

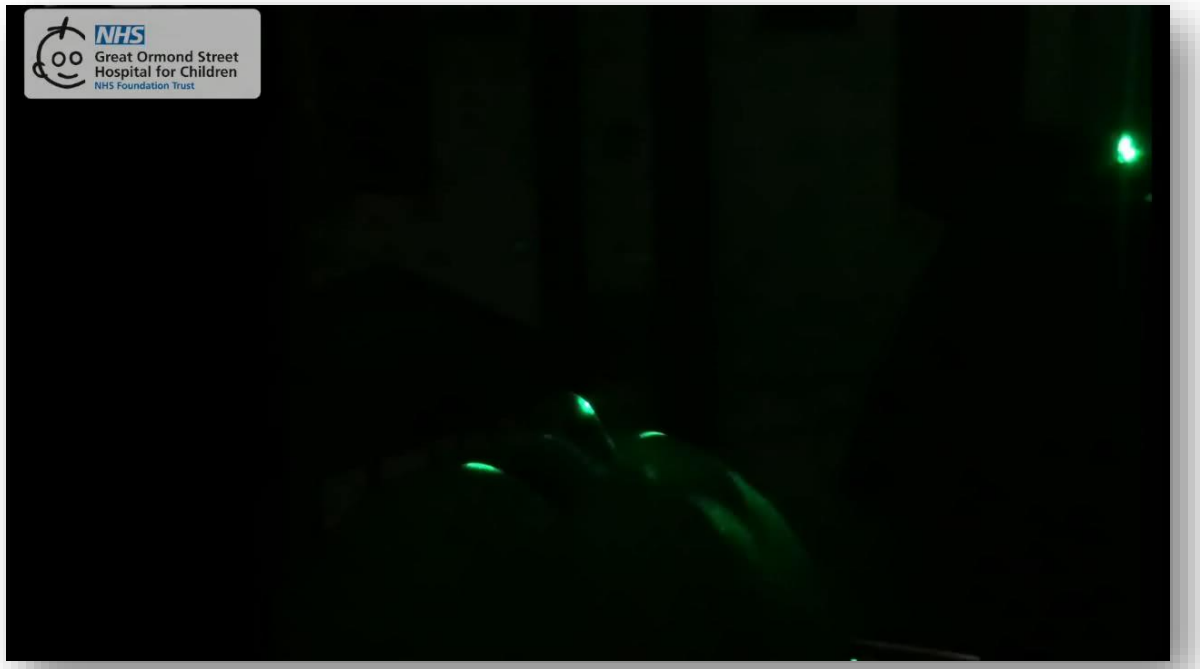
Improving collection efficiency

2 stage calibration & fillers
& viral concentration

Finding human ciliated cultures that replicate SARS-CoV-2 best



Higher throughput analysis of ciliary culture infections



Understanding aerosol generating procedures

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- Prof Chris O'Callaghan

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- Dr Claire Smith
- Dr Rob Hynds
- Francis Yongblah
- Dr Divya Shah

Sars-CoV-2 Research collaborators Imperial

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- Prof Wendy Savage
- Dr Ryan Thwaites

Porton Down

- Dr Allan Bennet
- Dr Barry Atkinson
- Dr Ginny Moore

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BBSRC

A question for you

If the next pandemic affected children, are we prepared?

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