



**Northumbria  
University**  
NEWCASTLE

# **100% SARS-CoV-2 Genome Detection in Saliva-Free Distal Airway Fraction Exhaled Breath.**

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Biodetection Technologies 2023

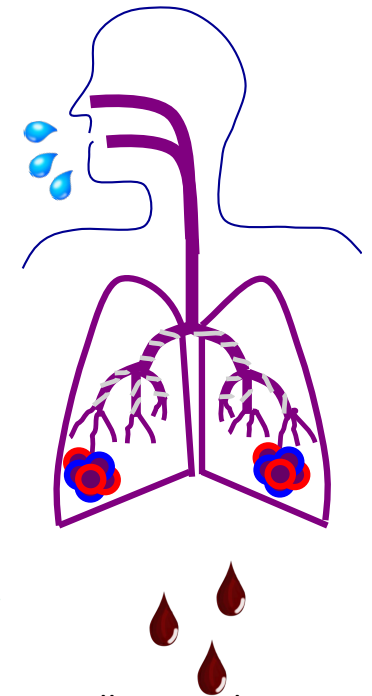
# Overview

- Pathogen sampling for respiratory health and biodefence.
- Non-invasive sampling of the deep lung: PBM-HALE™
  - Technology
  - Clinical study 1: No salivary/ambient contamination
  - Clinical study 2: SARS-CoV-2 detection & immune profiling
  - Forensic airborne DNA applications
- Future directions in security and medicine

# Pathogen sampling for respiratory health & biodefence in acute disease

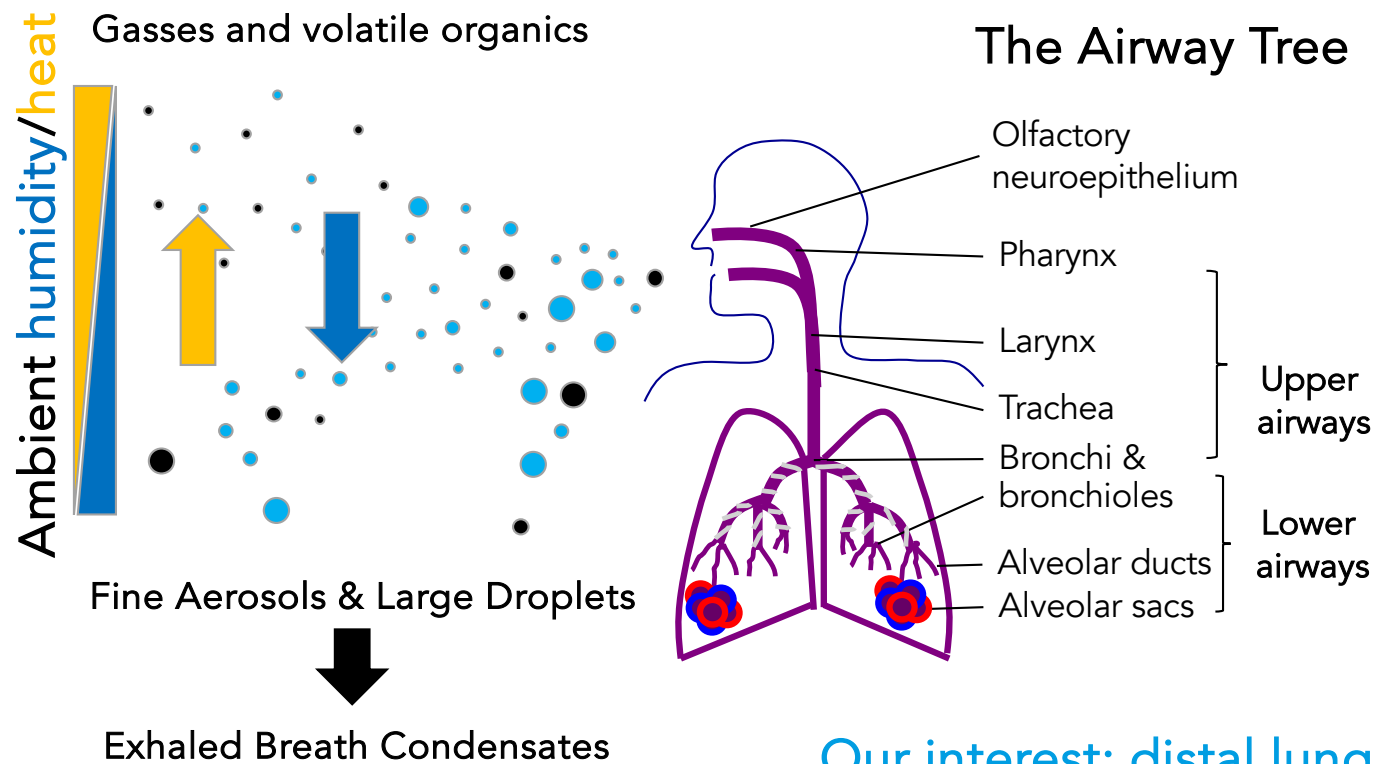
- Not all pathogens are detectable with swabs.
- Lower Respiratory Tract Infections:
  - Not all in sputum
  - Oral contamination
  - Invasive sampling needed

Specimen	Pathogen
Swabs, saliva	Influenza, Mumps, <i>Y. pestis</i> RSV, Poliovirus, Smallpox, <i>C. diphtheriae</i> SARS, MERS, SARS-CoV-2, <i>B. pertussis.</i> , Measles, NiV, HeV <i>N. meningitides</i> , MRSA
Sputum, BAL, Tracheal aspirate	<i>F. tularensis</i> , <i>S. pneumoniae</i> , Alphaviruses (e.g. VEE), <i>B. anthracis</i> , <i>Pseudomonas</i>
Peripheral blood	Q fever, filoviruses, <i>Brucella spp.</i> , CCHF, hantaviruses, bunyavirales, TB



Good, unreliable, poor detection. CDC Yellow Book 2024

# Exhaled breath as a sample



# Disease detection in breath & EBC



## Pre-COVID

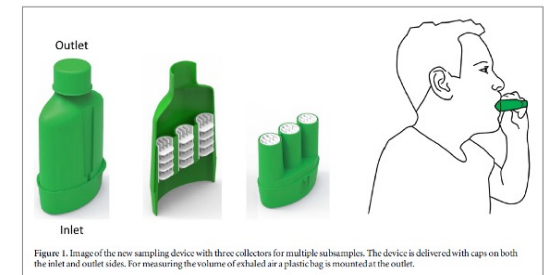
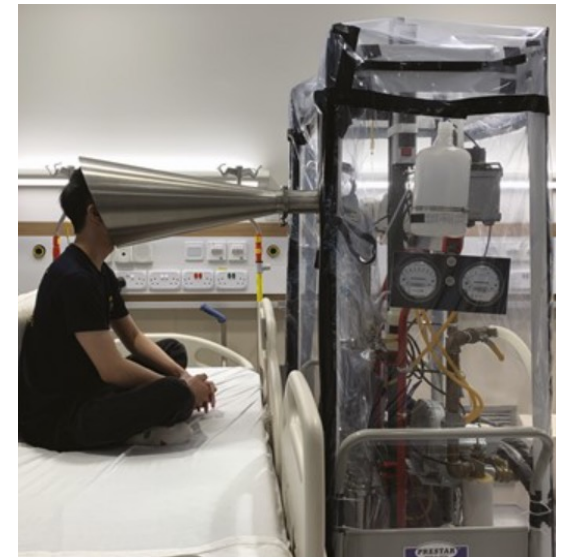
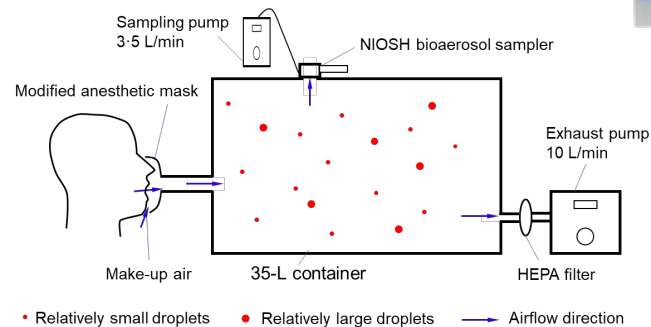
- Influenza aerosol RNA and infectious virus (Don Milton)
- Mixed reports from other groups

## Post-COVID

- Volatile Organic Compound signatures (EUA)
- Mixed reports on SARS-CoV-2 RNA and infectious virus in EBC

# Is SARS-CoV-2 exhaled?

- Ryan *et al* 2020: 66-93% +ve R-Tube, test dependent, n=16
- Feng *et al.* 2021, n=21, all -ve.



# Challenges to EBC clinical use

- Reproducibility.
- Contamination:
  - Saliva.
  - Ambient.
- Sample loss.
- Safety.
- Upper vs deep lung separation.

RTube™



Saliva contamination

EcoScreen™



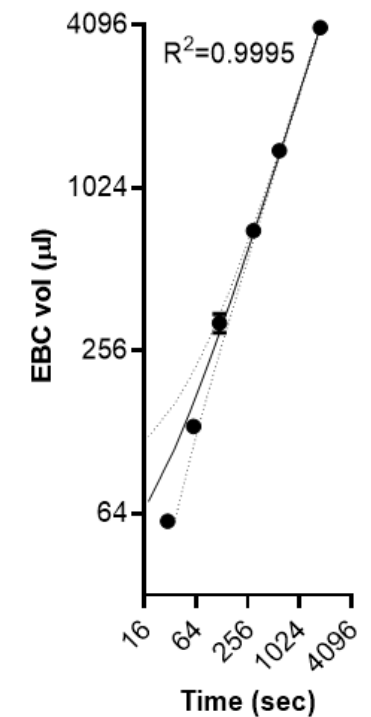
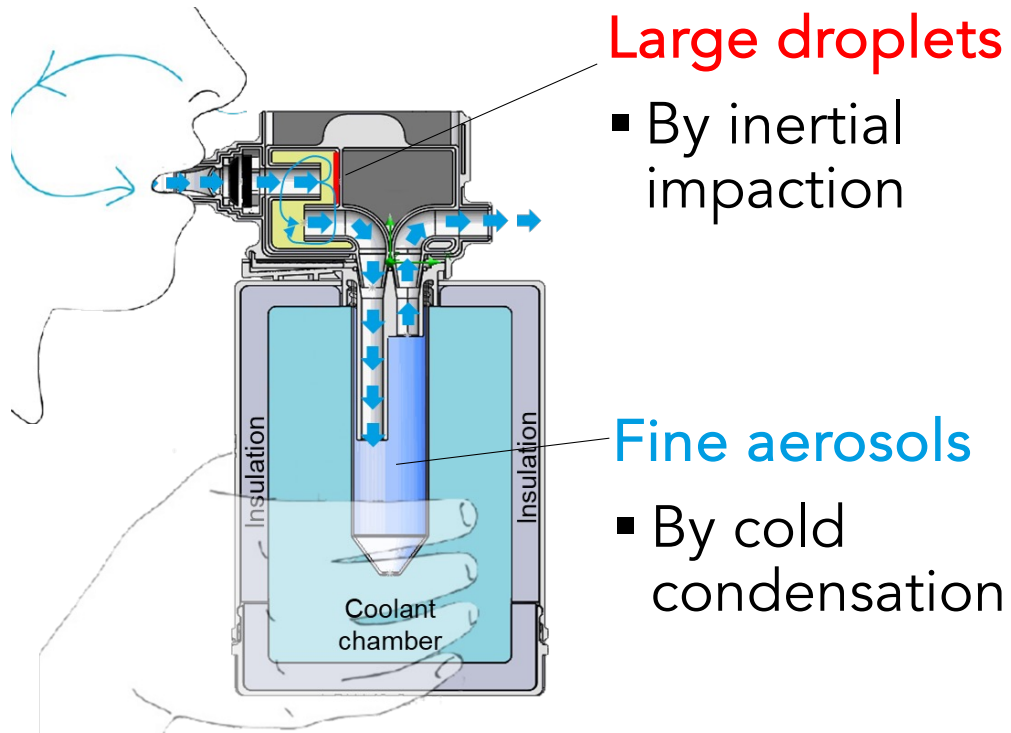
Sample lost in black tube  
17Kg + weight

# PBM-HALE™





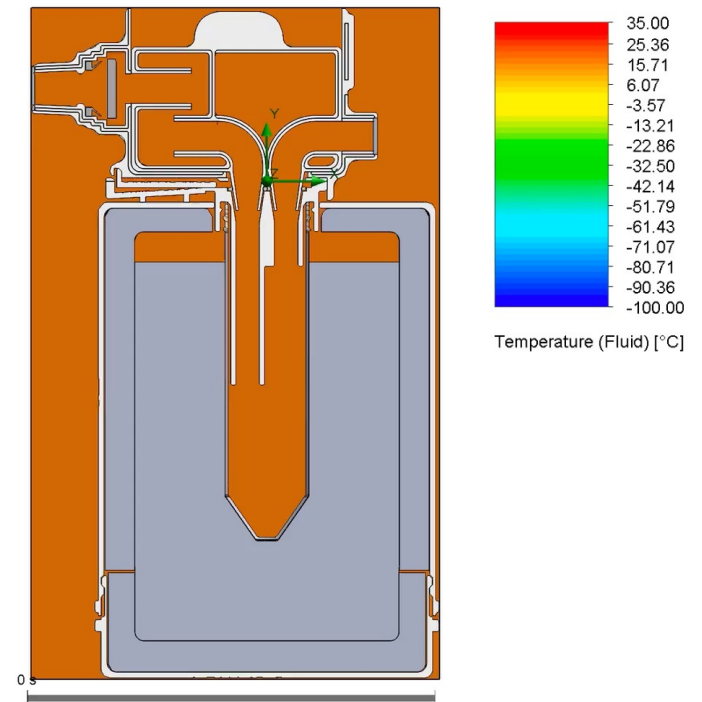
# PBM-HALE™: the platform collects



# Inhalation phase distal FA condensation

- 5 sec "stabilization"
- Tidal exhalation (0.5L/3 sec)
- No inhalation via the device
- Only the terminal 48mL of exhalation condensed

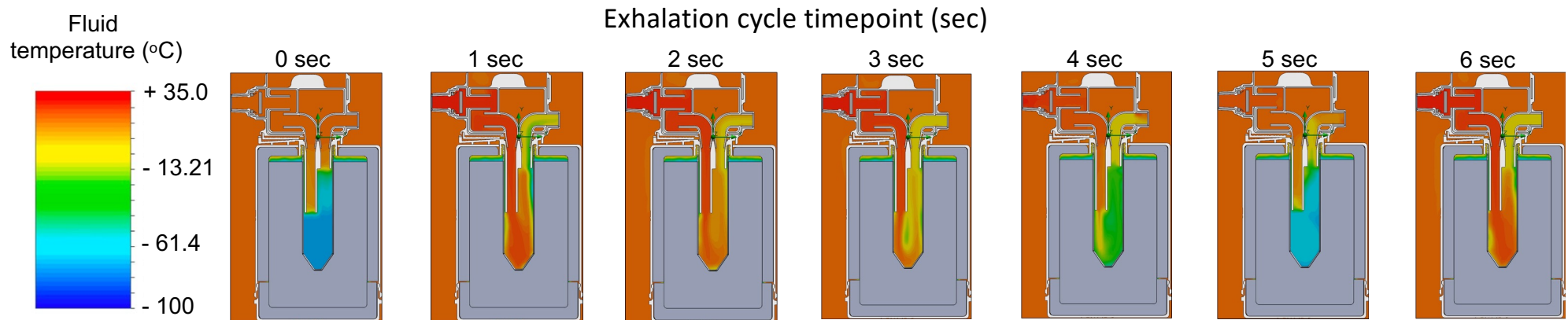
Time = 0 s



5 sec breathing cycle; 10,000 iteration convergence

Henderson J. *et al.* *MedRxiv* 2022; Data by Mr Saqib Ali, Dr Madeleine Combrink

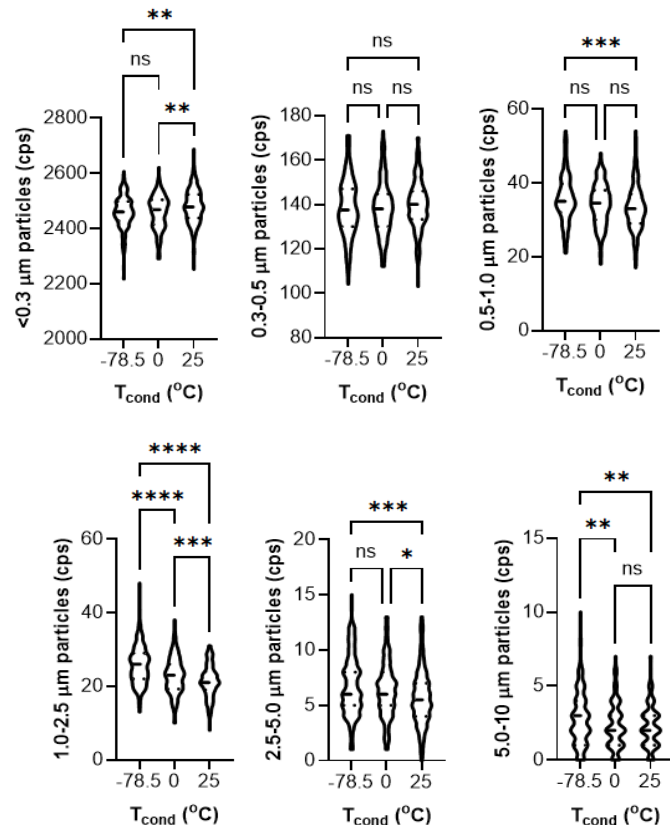
# Inhalation phase distal FA condensation



5 sec tidal breathing duty cycle; 10,000 iteration convergence

**Inhalation phase cooling condenses last 48mL of exhaled FA when inhalation is occurring, and exhalant is stationary**

# Exhaled particles swell due to condensation



- <0.5  $\mu m$  particle count drops
- >1  $\mu m$  particle count increases
- Effect maximal at  $-78.5^{\circ}C$
- Reproducible day-to-day
- No salivary amylase in FA (<1:1,750)

One way ANOVA w/ Holm-Šidák Multiple Comparisons

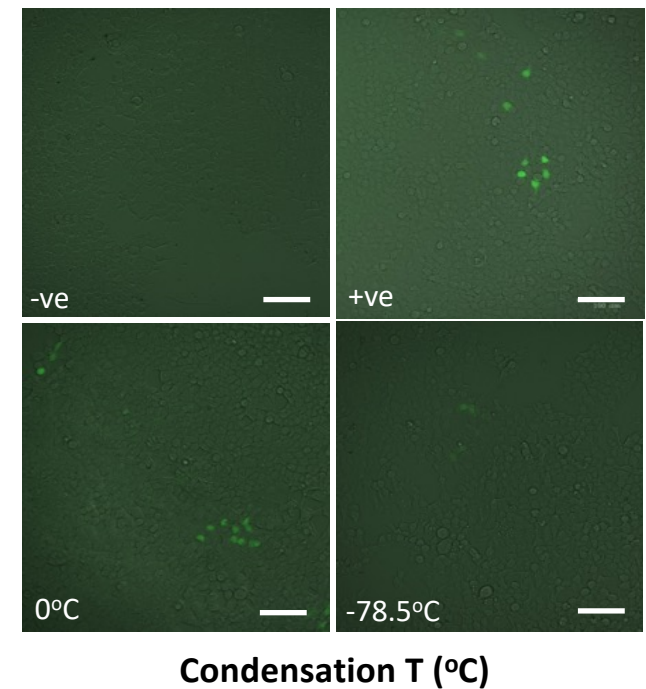
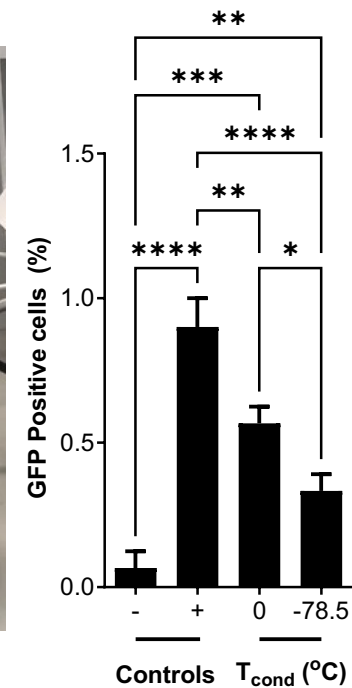
Henderson J. et al. MedRxiv 2022; Data by Mr Saqib Ali, Dr Theodora Mantso

# Nebulised virions are captured

GFP-encoding pseudotyped lentivirus



Condensate seeded at 0.013 MOI on 10,000 HEK-293T's  
FACS and fluorescent microscopy at 72 hrs



# Study 1: is SARS-CoV-2 in tidal EBC?



## Inclusion criteria:

- NP swab positive
- Within days 0-5 of symptoms

## Study size:

- n=60, 98% power, 10% +ve
- Interim data point: n=30
- N=12 outside inclusion criteria

## Samples:

- Tidal breathing
- 5-30 min
- Fine Aerosol, NP swab

## Outcome:

- No SARS-CoV-2 RNA in FA
- No environmental contamination

# Study 2: Does forced expiration help?



## Inclusion criteria:

- NP swab positive
- Within days 0-5 of symptoms

## Study size:

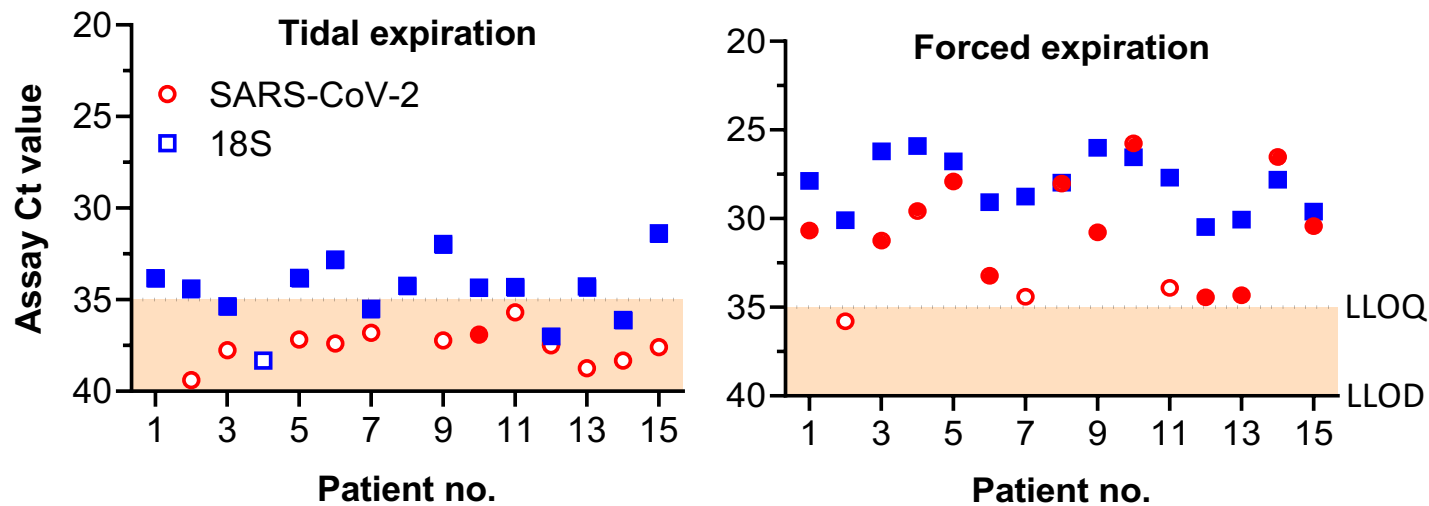
- n=30
- Interim data point: n=15

## Samples:

- Tidal breathing <30 min
- Forced expiration <15 min
- Paired Saliva, NP swabs, Fine Aerosols, Large Droplets

# Forced expiration increases yield

Total host RNA and SARS-CoV-2 RNA



100% of Forced Expiration samples positive, 1 min sample

90x increase in total RNA yield

CDC multiplex assay (N1, N2, RP) and 18S rRNA in 3 technical triplicates; No RP detected; empty shapes: <3 replicates, or only 1 assay fully positive (inconclusive)



# Effect of analysis protocol

## Study 1

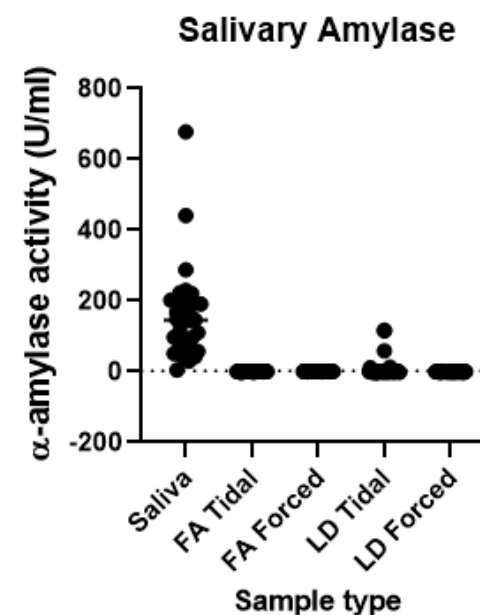
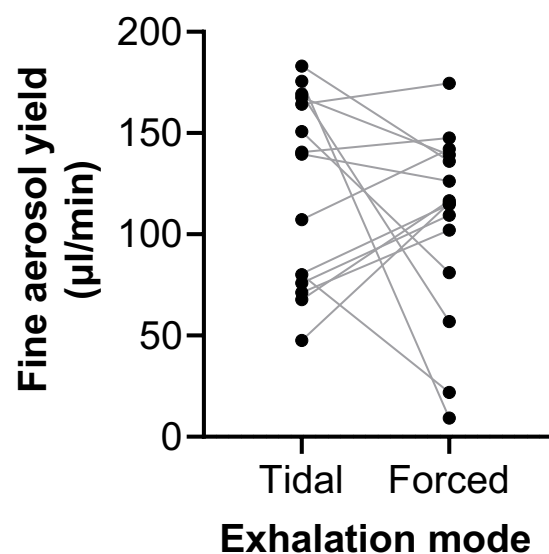
- Single RT-PCR per sample
- Internal control
  
- 1 inconclusive sample (Ct 38),  
negative on repeat

## Study 2

- Technical triplicate RT-PCRs
- Separate control reaction
- 8.7x more sample analysed =  
+2.95 Ct
- Tidal FA Ct =  $38.0 \pm 1.98$ ,  
mostly 1-2 of 3 replicates +ve

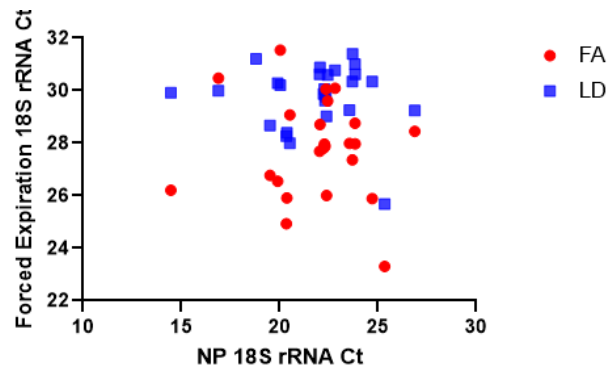
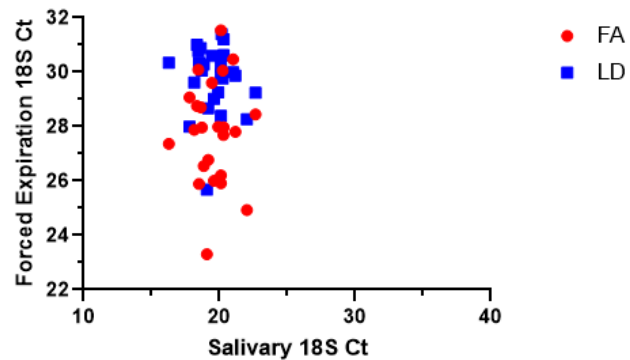
Viral RNA yields by tidal exhalation  
are at Poisson distribution levels

# Is forced expiration introducing salivary or nasal contamination?



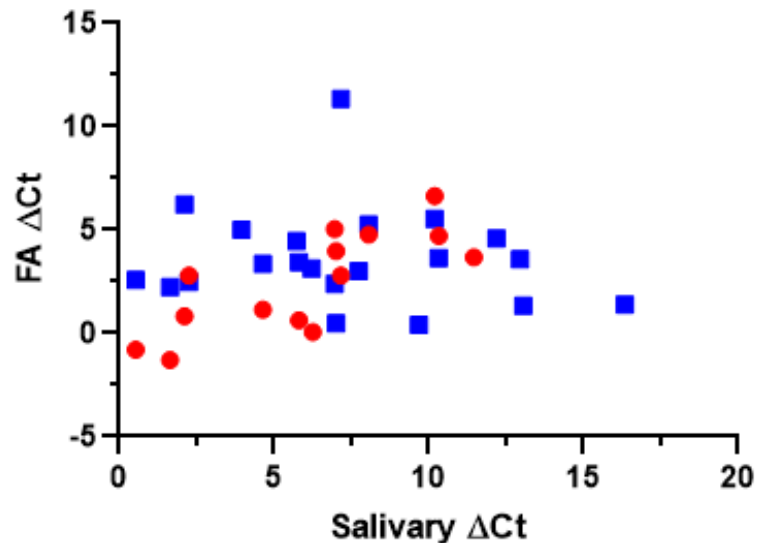
No extra FA volume due to saliva  
No salivary amylase detected

# Is forced expiration introducing salivary or nasal contamination?



- No 18S rRNA (total RNA) correlation between:
  - Saliva or NP swab vs LD
  - Saliva or NP swab vs FA
- Total RNA is not due to saliva contamination
- Any FA SARS-CoV-2 load is from the lower airways

# Are forced expiration introducing viral load contamination?

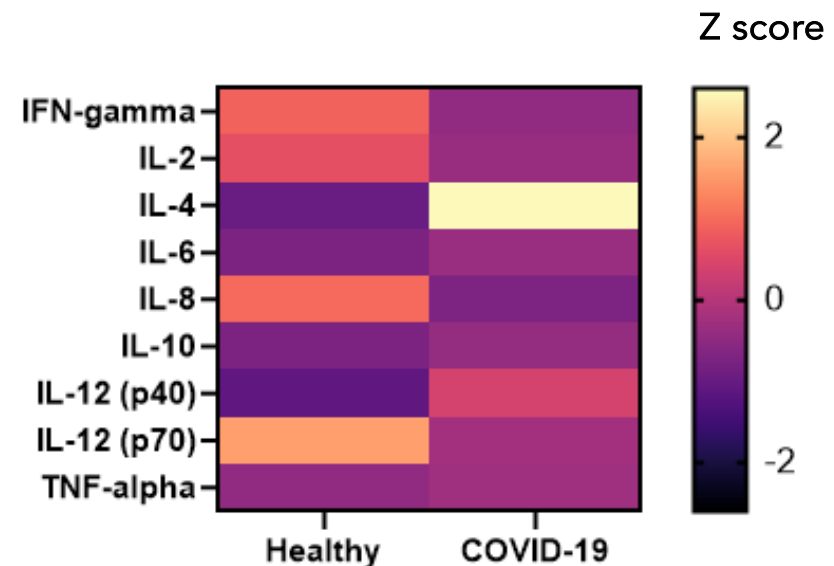
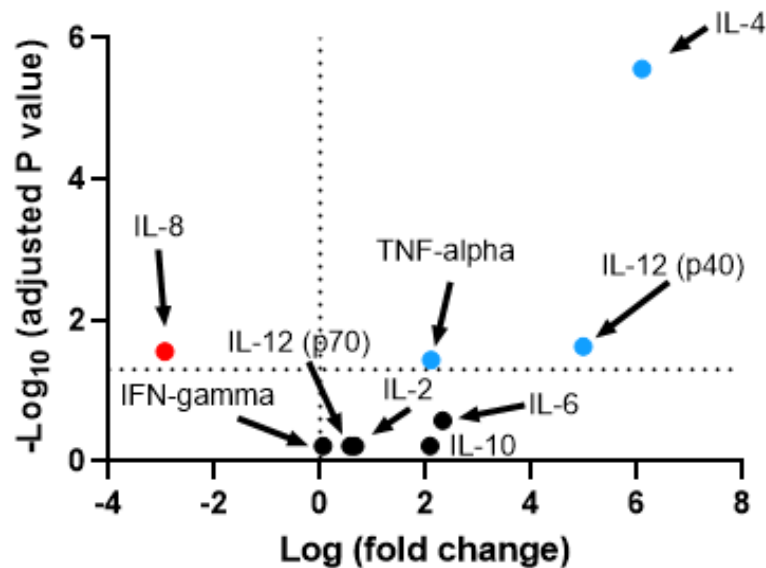


● Forced Expiration  $R^2 = 0.7802, p = 0.0015$   
■ Tidal Breathing NS

- Salivary but not NP load correlates to FA load
- 85.6x higher normalized viral loads vs saliva
- Only 4 FA samples have lower normalized viral loads

Viral loads are comparable but higher in the lower lung

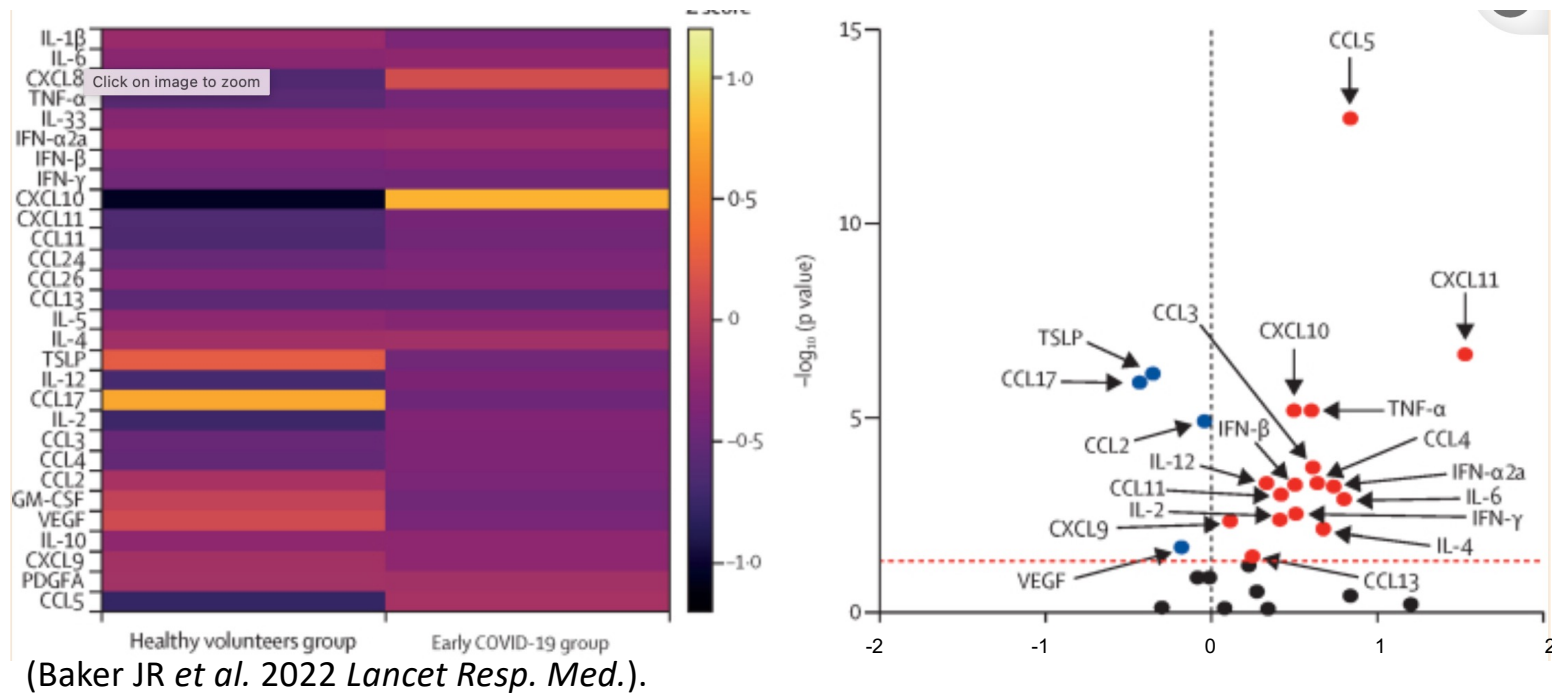
# Can we detect inflammation?



10 sec sample equivalent: 0.02 mL analysed only

All cytokine changes observed in Tidal Breath Fine Aerosols in acute COVID-19 are also reported in independent study using invasive sampling (Baker JR *et al.* 2022 *Lancet Resp. Med.*). N=10 healthy participants; N=15 acute COVID-19 cases. Mann-Whitney test with multiple comparison corrections. Coloured points represent statistically significant changes.

# Comparable response in the nose



All common cytokines change in similar ways  
between the two studies, but intensity is higher in the lung

# Conclusions & Future Work

## Conclusions

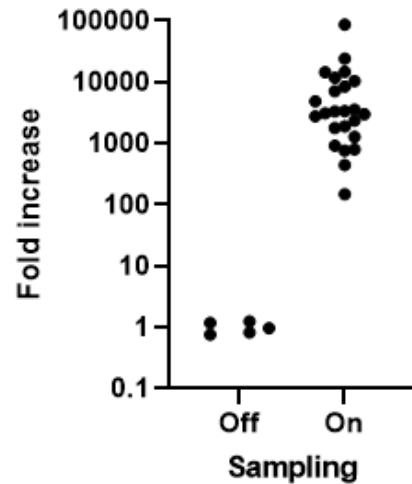
- Contamination-free aerosols from the deep lung.
- Forced expiration increases RNA and pathogen yields
- Pathogen detected in all QC pass samples
- Distal airway immune responses can be quantified, non-invasively

## Future Work

- Utility for other:
  - Biodefence pathogens
  - Diseases (e.g. cancer, asthma)
  - Medication monitoring
- Use in forensics?
  - Airborne DNA
  - Illicit drug use

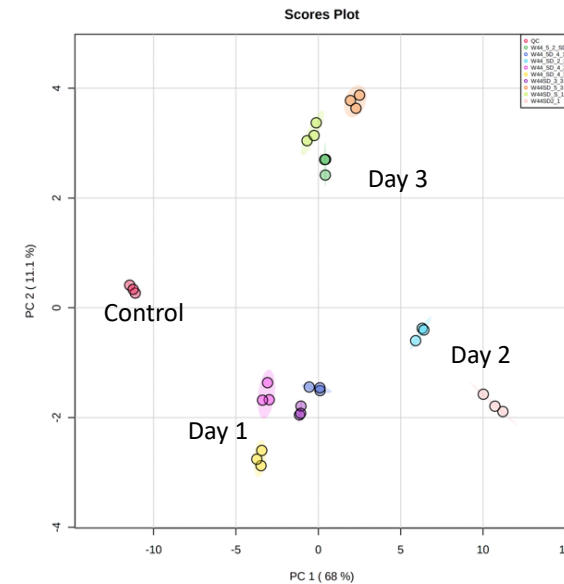
# Forensic uses? Breaking data...

## Active sampling human eDNA detection



Human 18S gene detected after 30min sampling using PBM-HALE™ fitted with active sampling fan module within 1m of subject: **3,492x signal** (ON) difference vs **background** (OFF) levels in reagents (n=30 tests).

## Metabolite, medication, and illicit drugs



Day to day and intra-day metabolite reproducibility by HILIC-MS using 2 min samples. Blinded detection of **cuscohygrine**, **THC**, **escitalopram**, **esomeprazole**, even 3 days after drug abuse.



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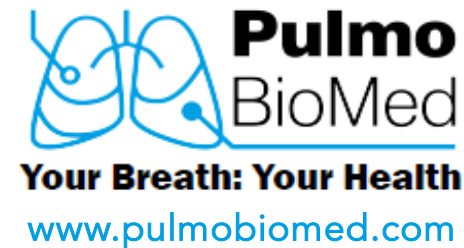
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