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Gasses, Volatiles, Cytokines, Mutations:

Personalising Lung Medicine through Breath Diagnostics.

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Overview

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- 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

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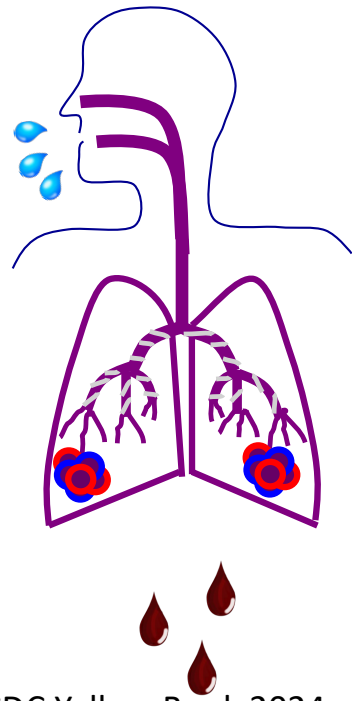
- Not all pathogens are detectable with swabs.
- Lower Respiratory Tract Infections:
  - Not all in sputum
  - Oral contamination
  - Invasive sampling needed

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



Good, unreliable, poor detection. CDC Yellow Book 2024

Exhaled breath as a sample

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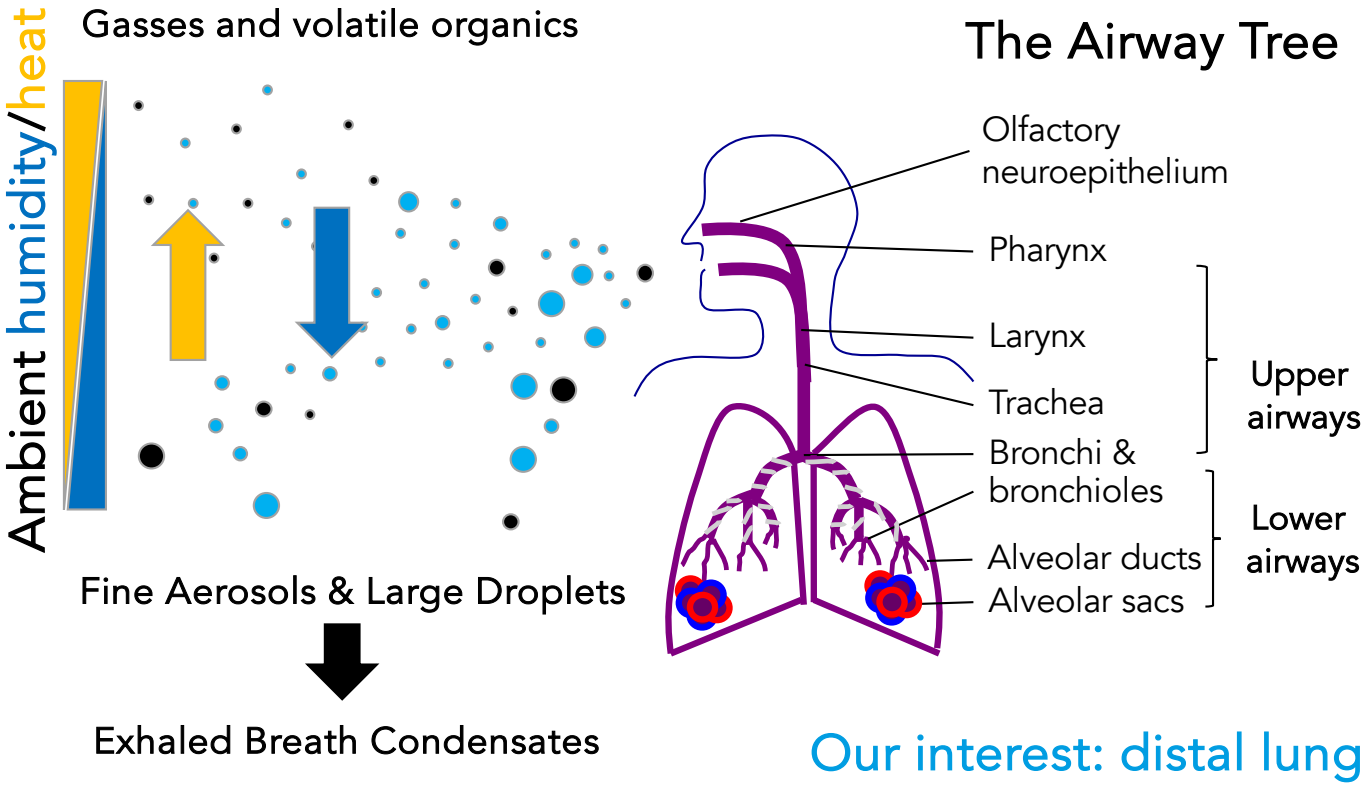

Our interest: distal lung fine aerosols

Disease detection in breath & EBC

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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?

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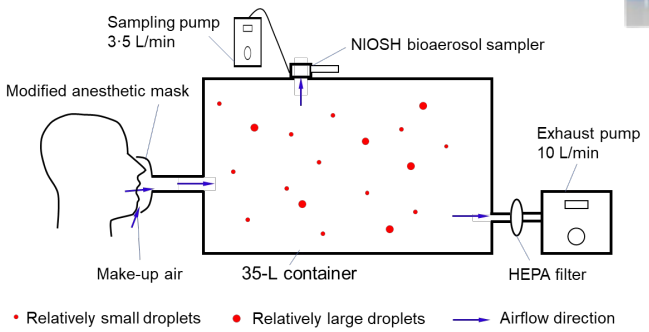
- Ryan *et al* 2020: 66-93% +ve R-Tube, test dependent, n=16
- Feng *et al*. 2021, n=21, all -ve.



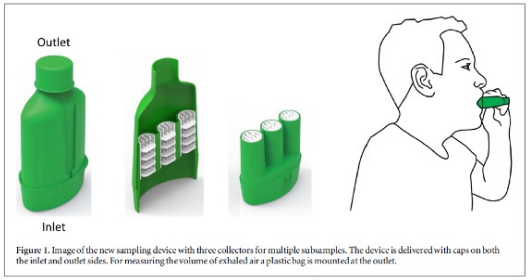


Figure 1. Image of the new sampling device with three collectors for multiple subsamples. The device is delivered with caps on both the inlet and outlet sides. For measuring the volume of exhaled air a plastic bag is mounted at the outlet.

Challenges to EBC clinical use

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

RTube™

Saliva contamination

EcoScreen™

Sample lost in black tube
17Kg + weight

Horváth, I., *et al*, 2017. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *European Respiratory Journal*, **49(4),** p.1600965.

PBM-HALE™

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PBM-HALE™: the platform collects

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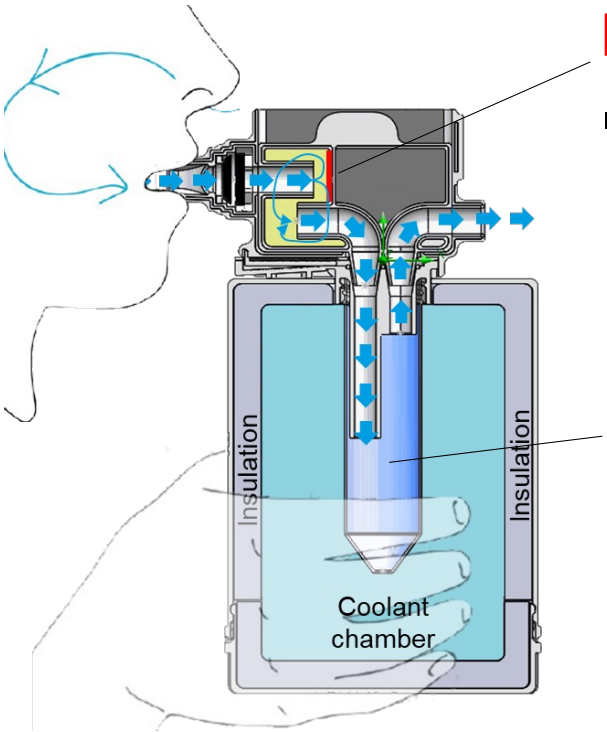
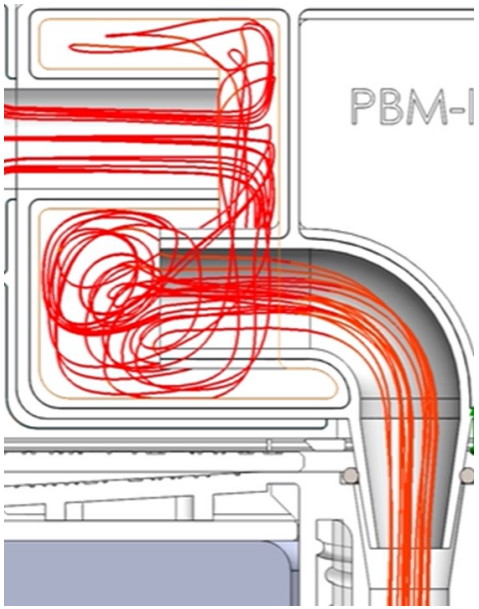


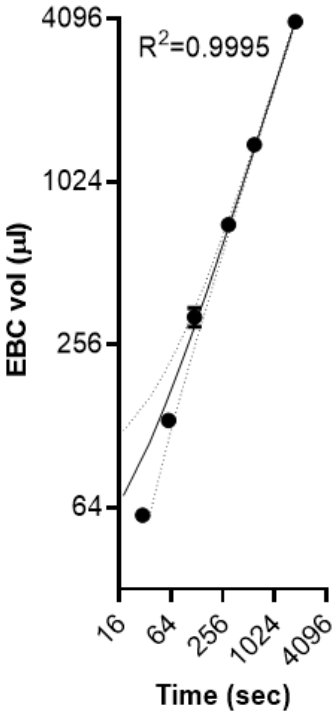
Large droplets

- By inertial impaction

Fine aerosols

- By cold condensation





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

Inhalation phase distal FA condensation

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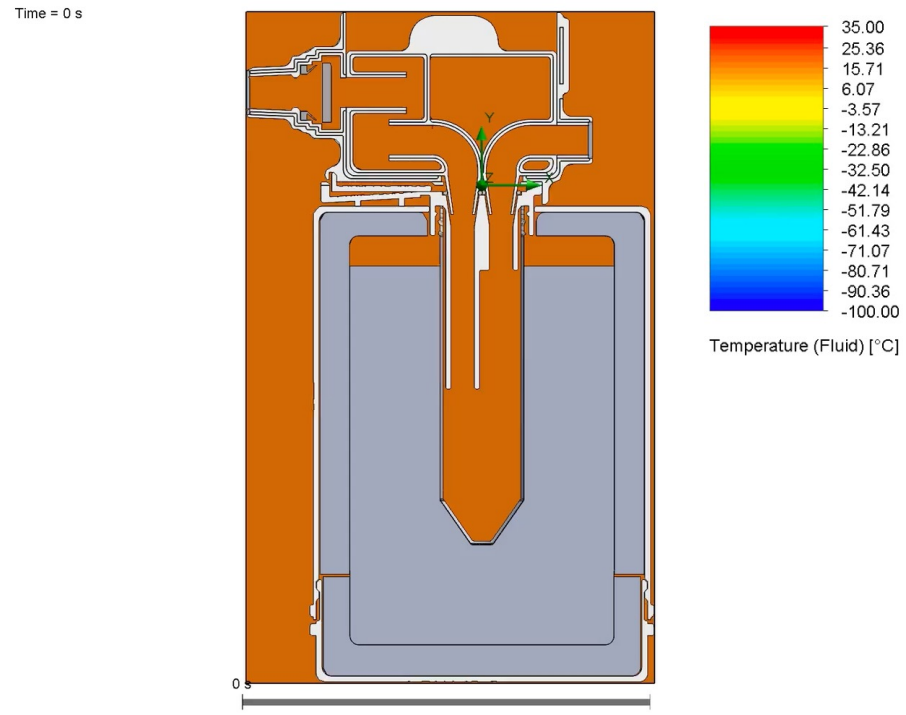
- 5 sec “stabilization”
- Tidal exhalation (0.5L/3 sec)
- No inhalation via the device
- Only the terminal 48mL of exhalation condensed



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

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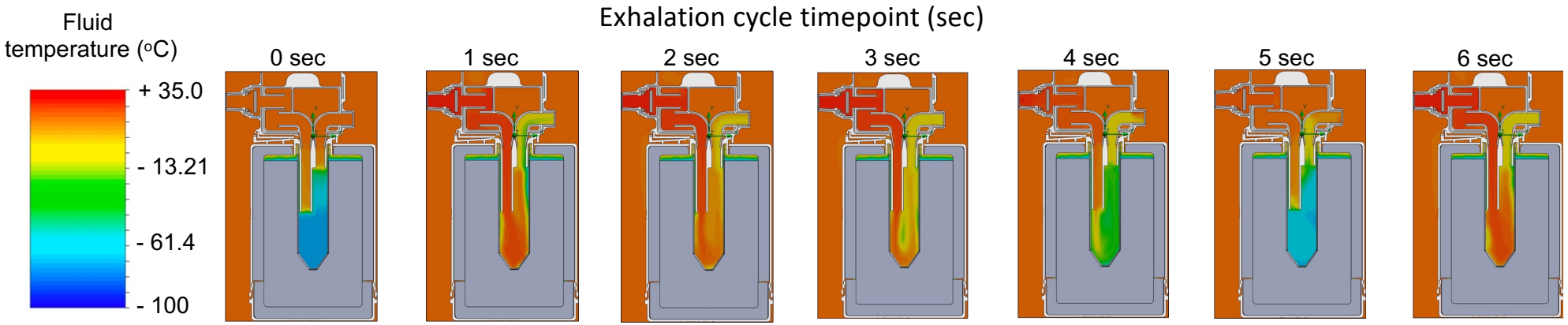

5 sec tidal breathing duty cycle; 10,000 iteration convergence

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

Exhaled particles swell due to condensation

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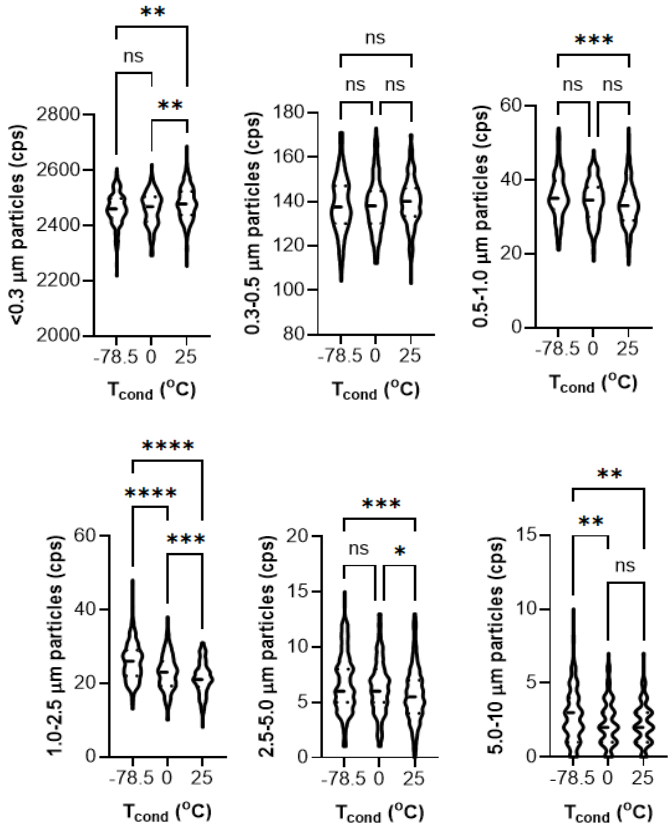

- <0.5 um particle count drops
- >1 um particle count increases
- Effect maximal at -78.5°C

- Reproducible day-to-day
- No salivary amylase in FA (<1:1,750)

One way ANOVA w/ Holm-Šídák Multiple Comparisons

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

Nebulised virions are captured

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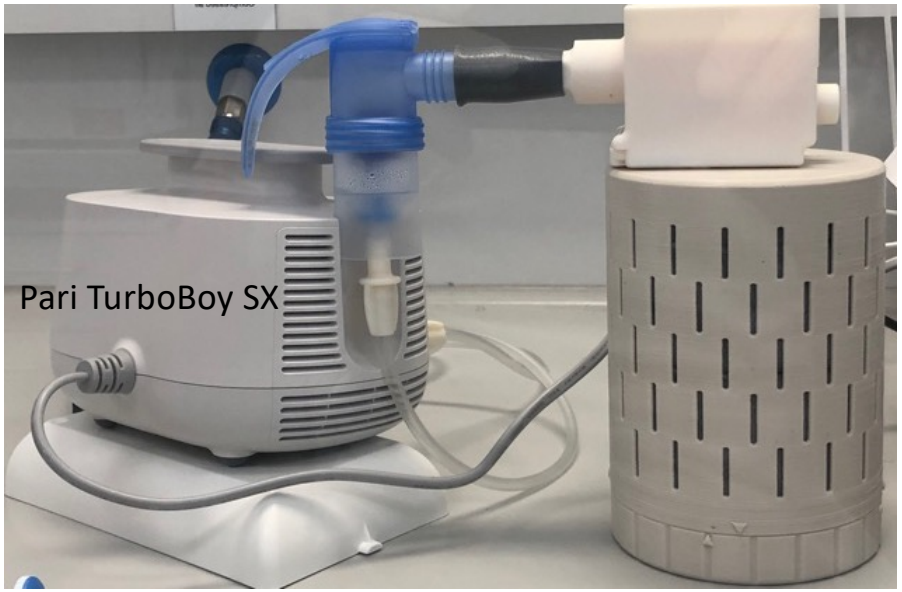
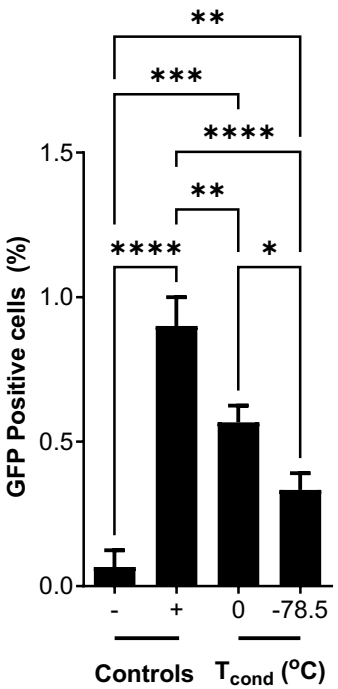
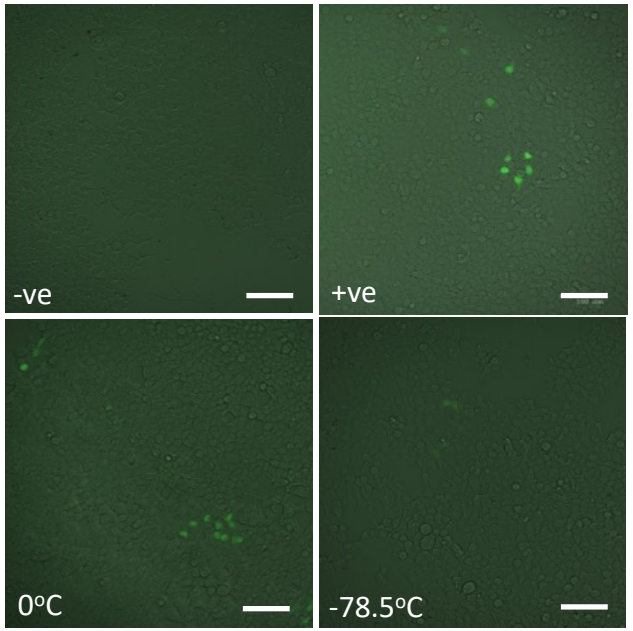
GFP-encoding pseudotyped lentivirus







Condensation T (°C)

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?

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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

Dr P. Katsaounou and G. Magiorkins groups, Athens, Greece

Study 2: Does forced expiration help?

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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

Prof. M.M. Teixeira and Dr R.S Aquiar groups, UF Minas Gerais, Belo Horizonte, Brazil

Forced expiration increases yield

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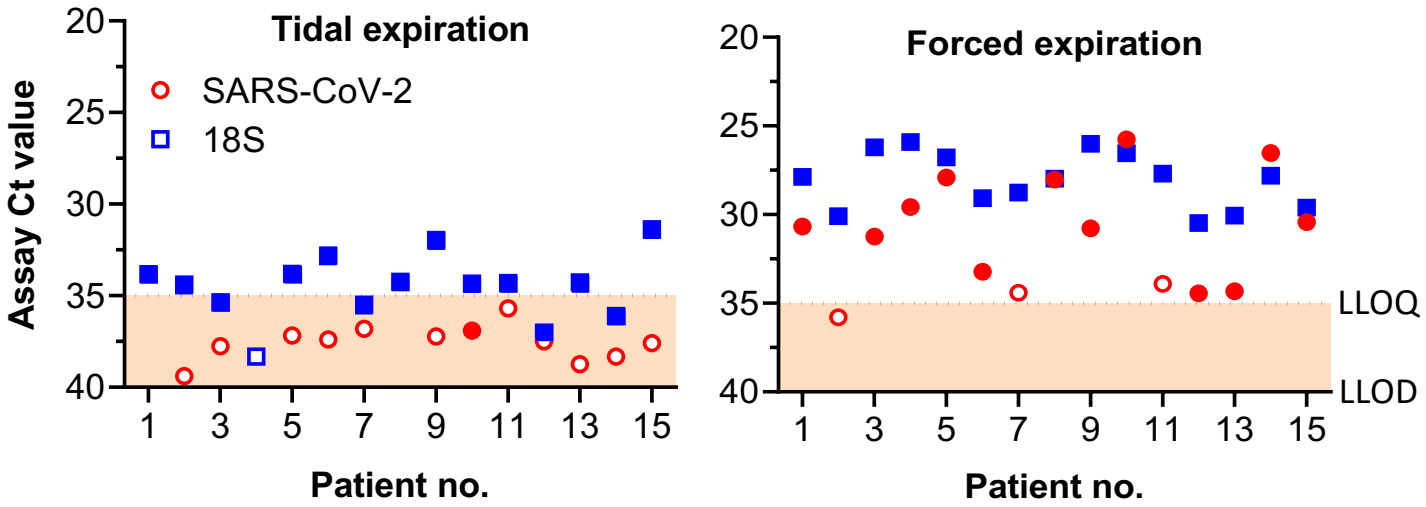
Total host RNA and SARS-CoV-2 RNA



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

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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 ±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?

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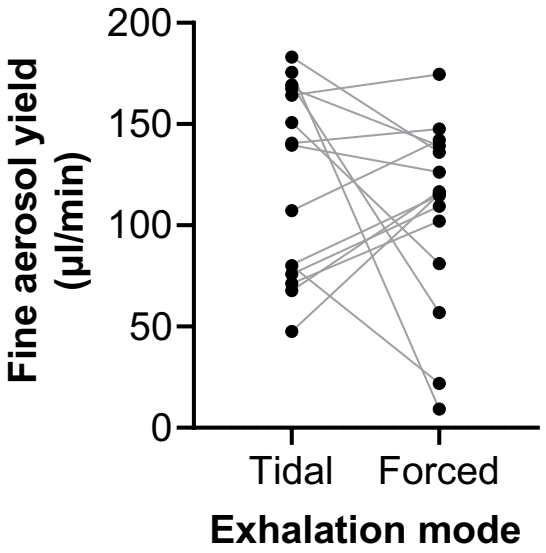

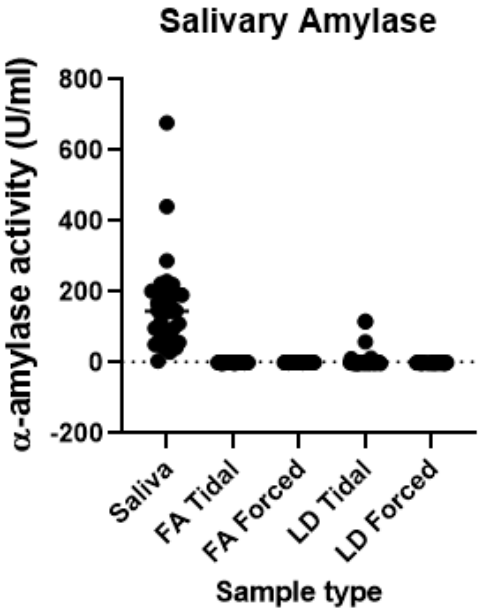

No extra FA volume due to saliva

No salivary amylase detected

Is forced expiration introducing salivary or nasal contamination?

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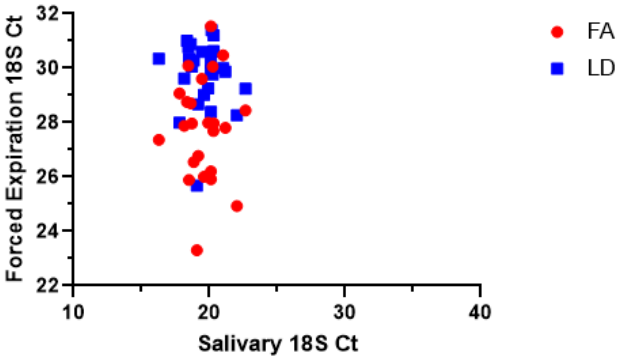

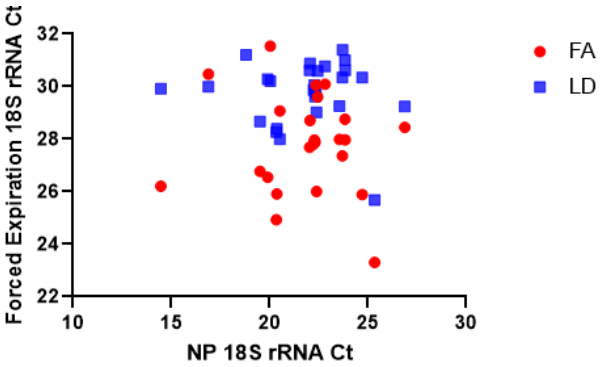

- No 18S rRNA (total RNA) correlation between:
  - Saliva or NP swab vs LD
  - Saliva or NP swab vs FA
- Total RNA is not due to saliva contamination
- Any FA SARS-CoV-2 load is from the lower airways

Are forced expiration introducing viral load contamination?

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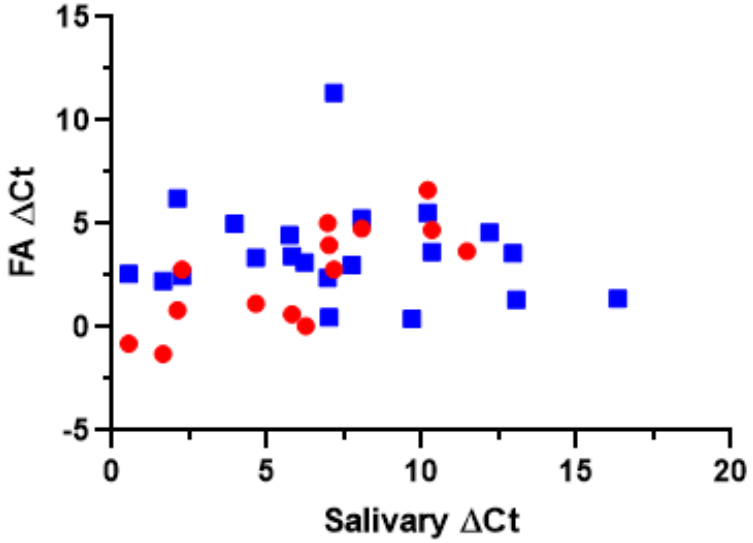

- Forced Expiration $R^2 = 0.7802$, $p = 0.0015$
- Tidal Breathing NS

- 85.6x higher FA viral loads vs saliva
- Only 4 FA samples have lower viral loads

Viral loads are *higher* in the lower lung vs saliva when normalized for RNA extraction

Can we detect lower lung inflammation?

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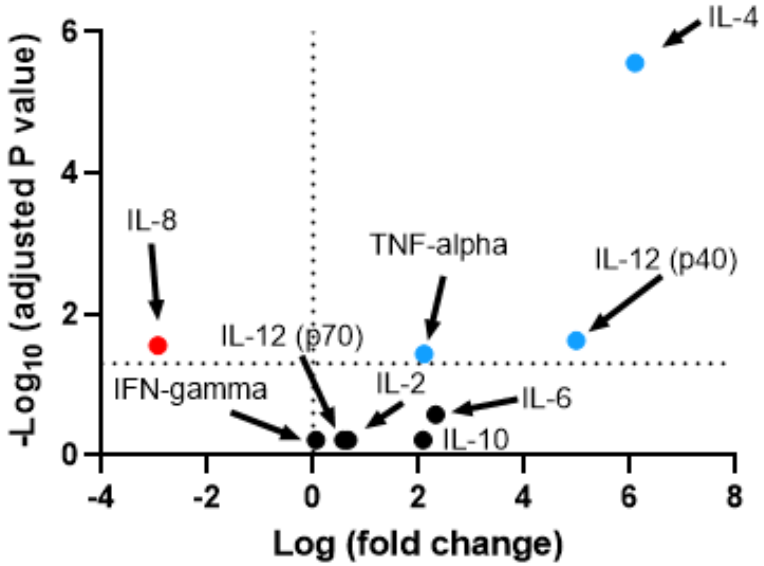

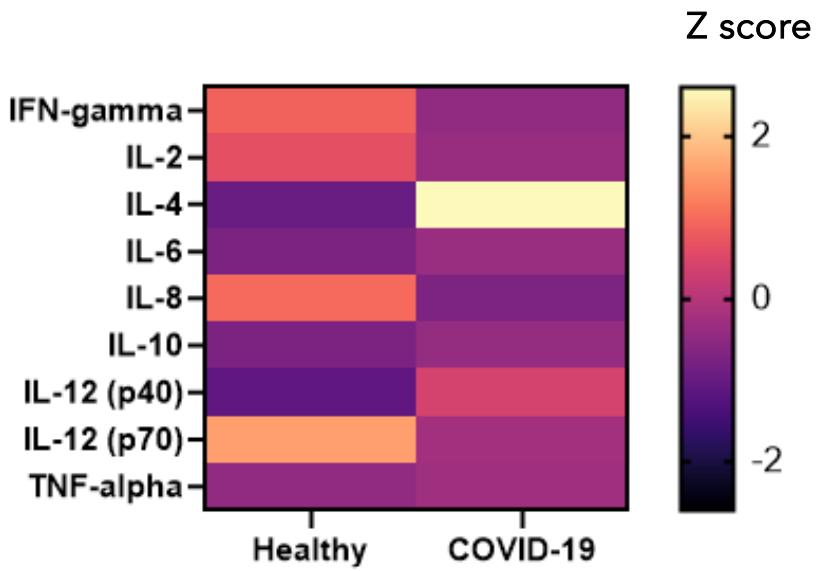

10 sec sample equivalent: 0.02 mL analysed only

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

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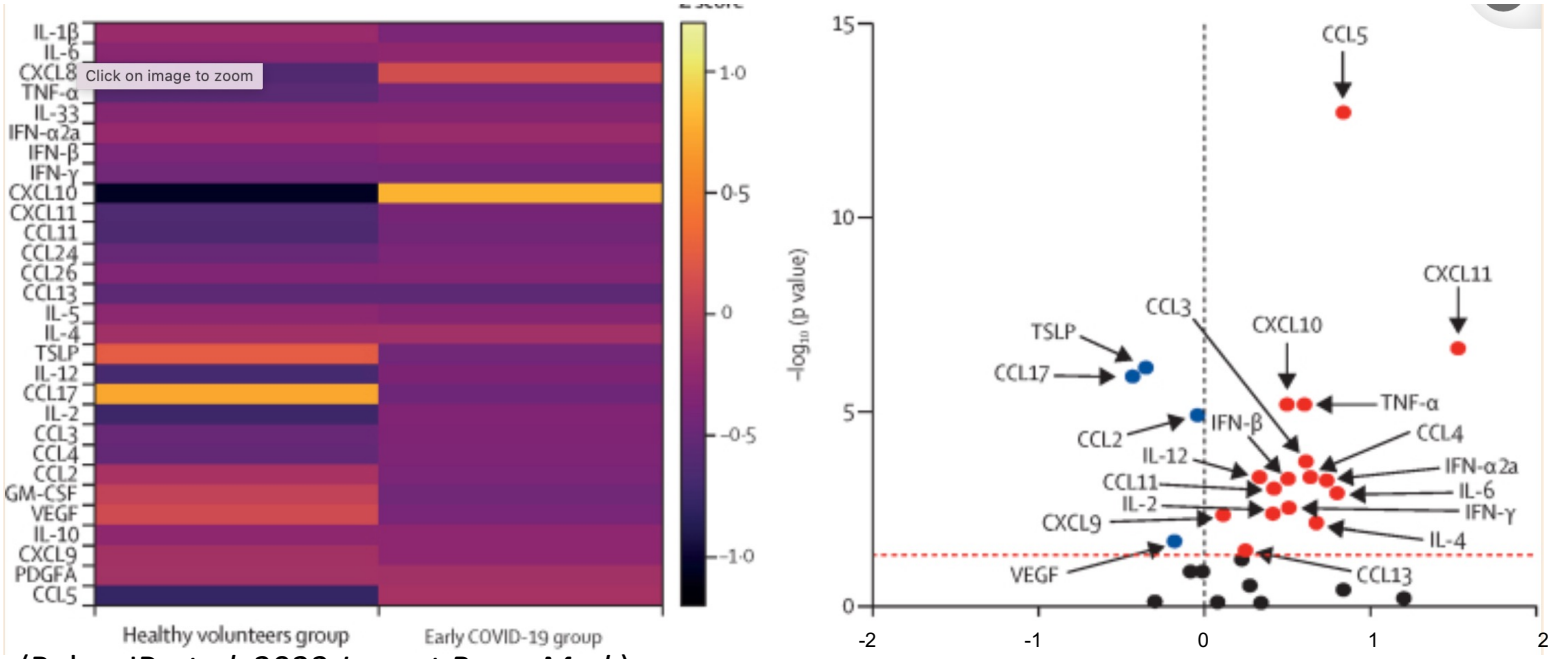

(Baker JR *et al.* 2022 *Lancet Resp. Med.*).

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

Conclusions & Future Work

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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

Henderson J. *et al.* MedRxiv 2022

Forensic uses? Breaking data...

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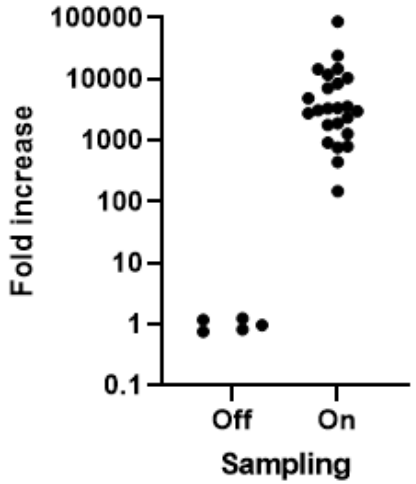
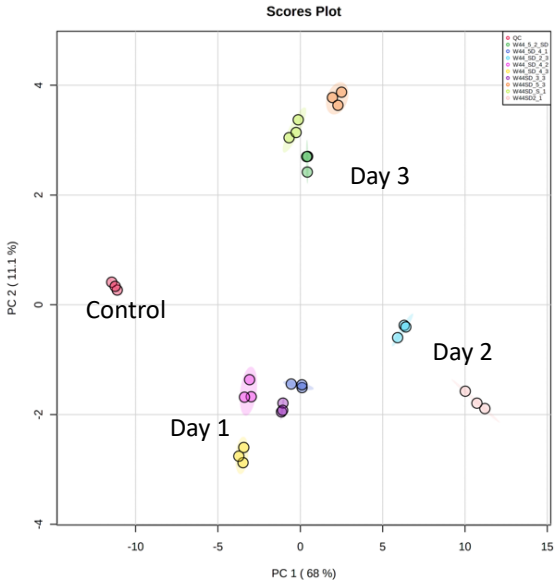
Active sampling human eDNA detection



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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