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# Deep lung fine aerosols as a novel sample for translational biomarker discovery

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Oxford Global NextGen Omics 2022

# Overview

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- Exhaled breath
  - Composition
  - Anatomy
  - Physics
- Clinical use: progress to date
  - Volatile compounds
  - Condensates
- The impact of COVID-19 research
- Future directions

# Exhaled Breath

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## Breath is a mix of:

- Volatile compounds
- Aerosols.
- Non-volatile biological molecules.

## Biomarkers:

- Lung infections.
- Liver diseases.
- Multiple cancers:
  - Blood.
  - Breast.
  - Brain.

# Exhaled breath: origin, content

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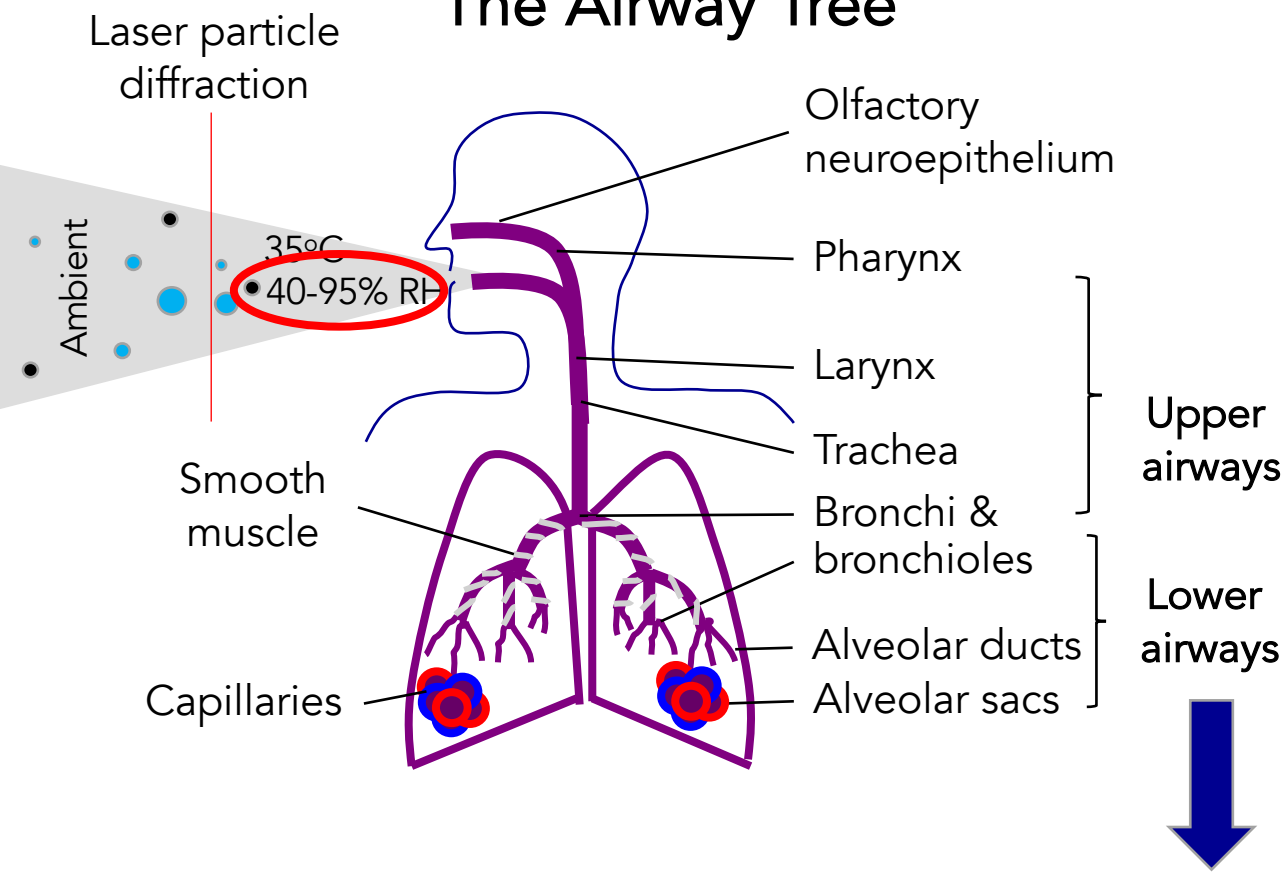
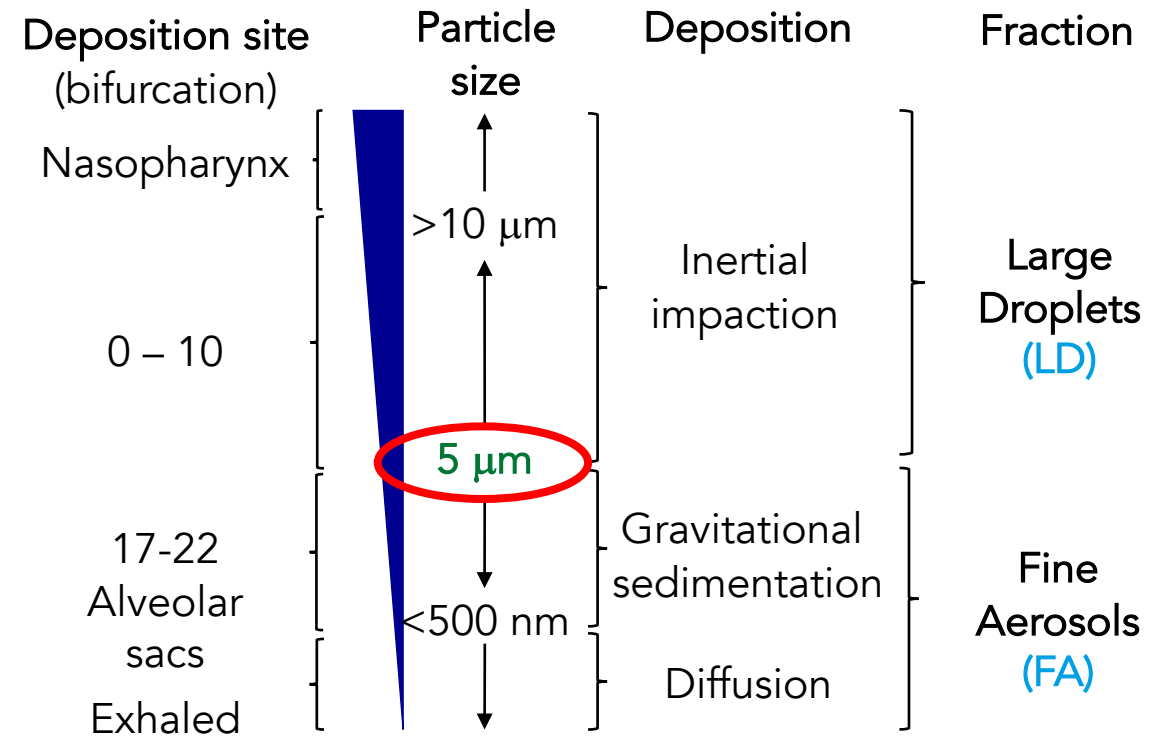
## The Airway Tree




## Inhaled particles




Airway lining fluid droplets
Molecular water

Moschos SA *et al. Pharm. Ther.* 2017

# Exhaled breath: origin, content

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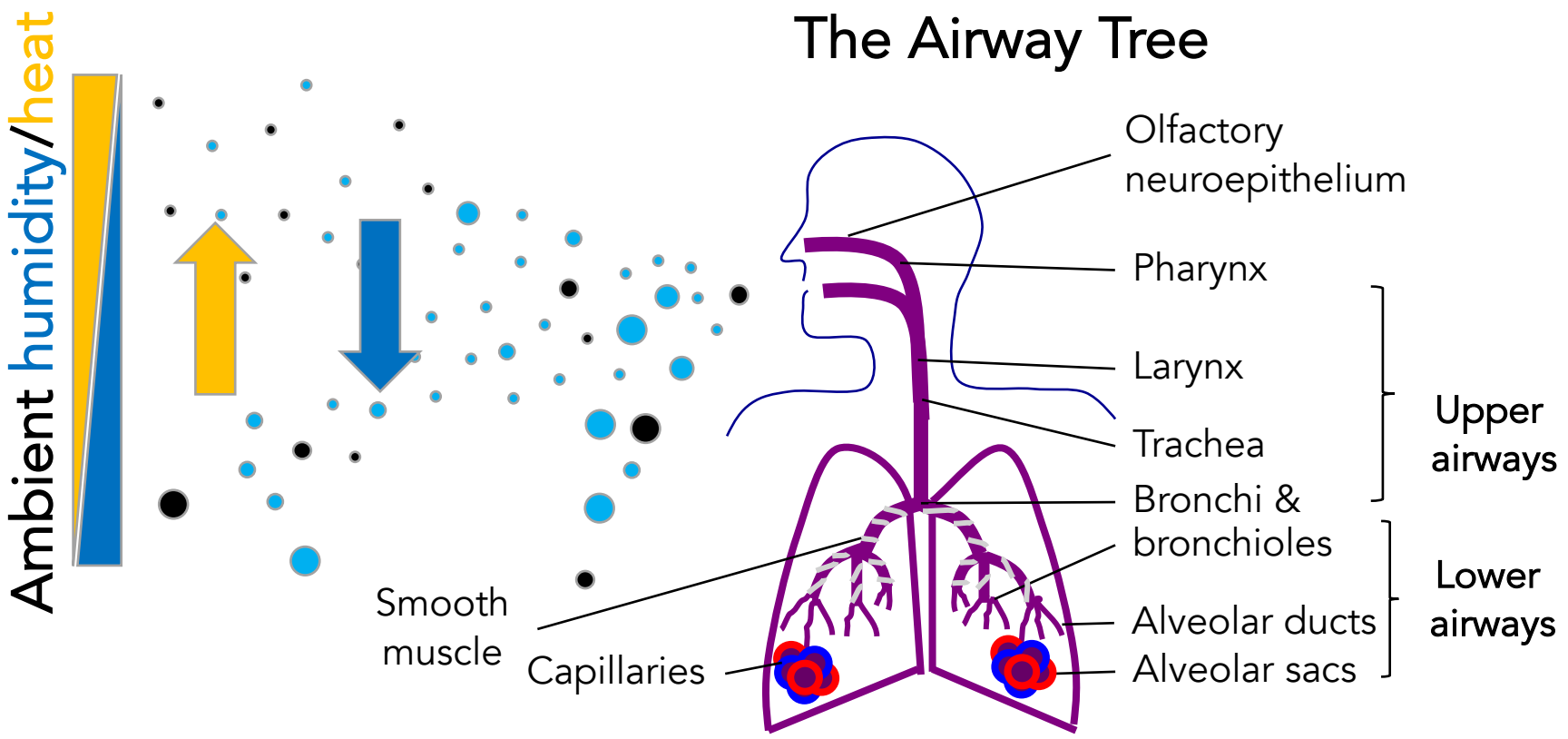

# Exhaled Breath Condensation (EBC): physics

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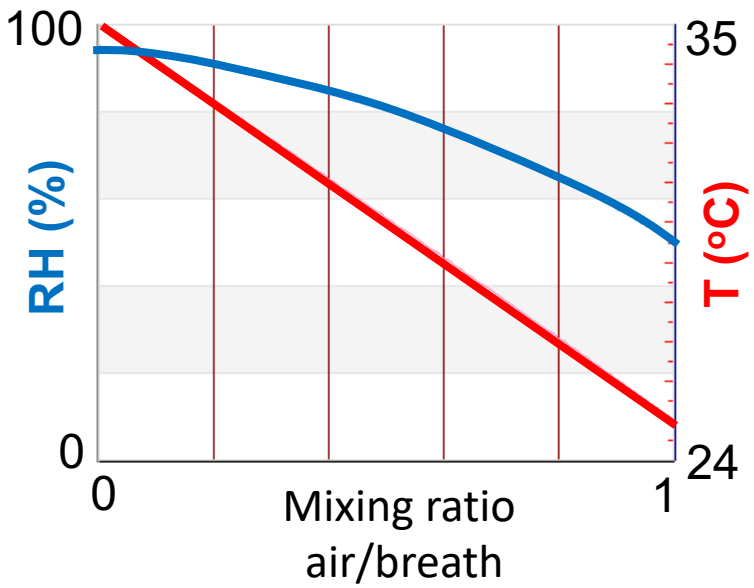
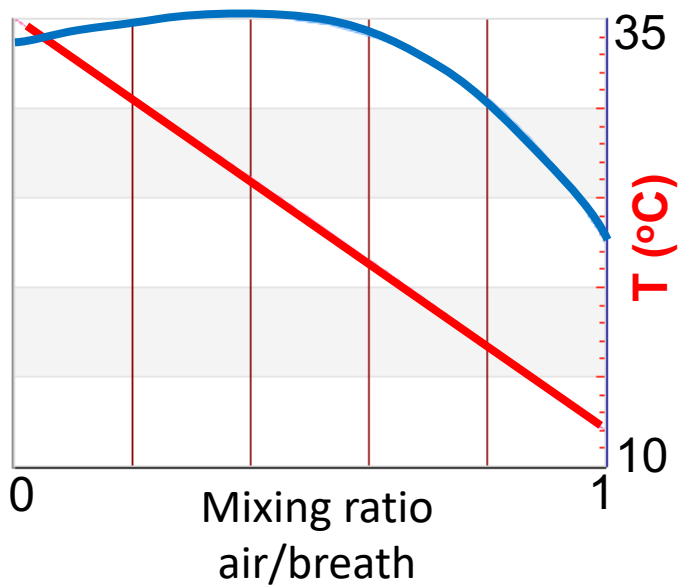
## Ambient conditions

**25°C, 50% RH**



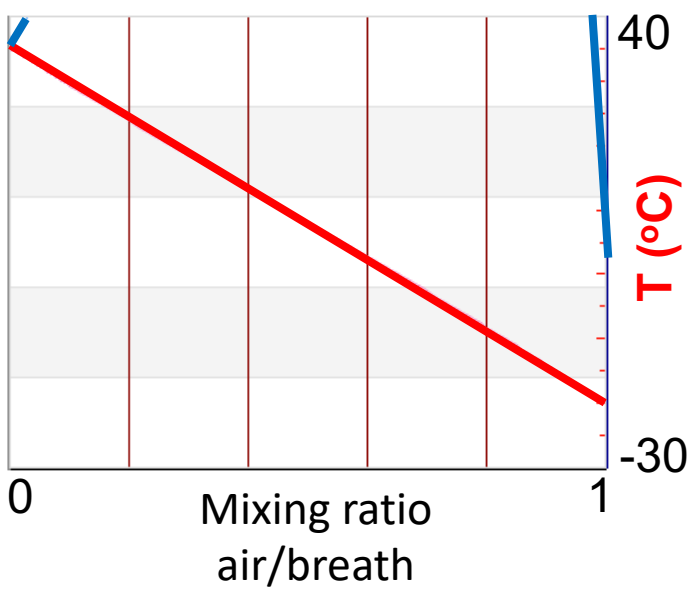

**No condensation**

**12°C, 50% RH**




**0.19 g/kgr water/air**

**-20°C, 50% RH**




**11.1 g/kgr water/air**

Dry ice: -78.5°C condensation yields 22.3g/kg @ 0-100% RH; tidal breath vol ~0.5 L; air density is ~0.13% of water; 1 kg air ~ 2hrs

# Clinical adoption: Volatile compounds

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## Nitric Oxide

- Produced in response to inflammation
- Kharitonov *et al.* 1994 Lancet
- 2003 FDA approval
- 2017 NICE adoption

## $^{13}C$ compounds

- *H. pylori*
- Gastric emptying
- Disaccharide malabsorption
- Small bowel bacterial overgrowth

## Multi-analyte

- COVID-19 tests
- Singapore, NL, USA EUA
  - High variation reports (UK)
  - Specificity unclear
  - NL field use failed
  - Limited news otherwise

# Fractional exhaled NO

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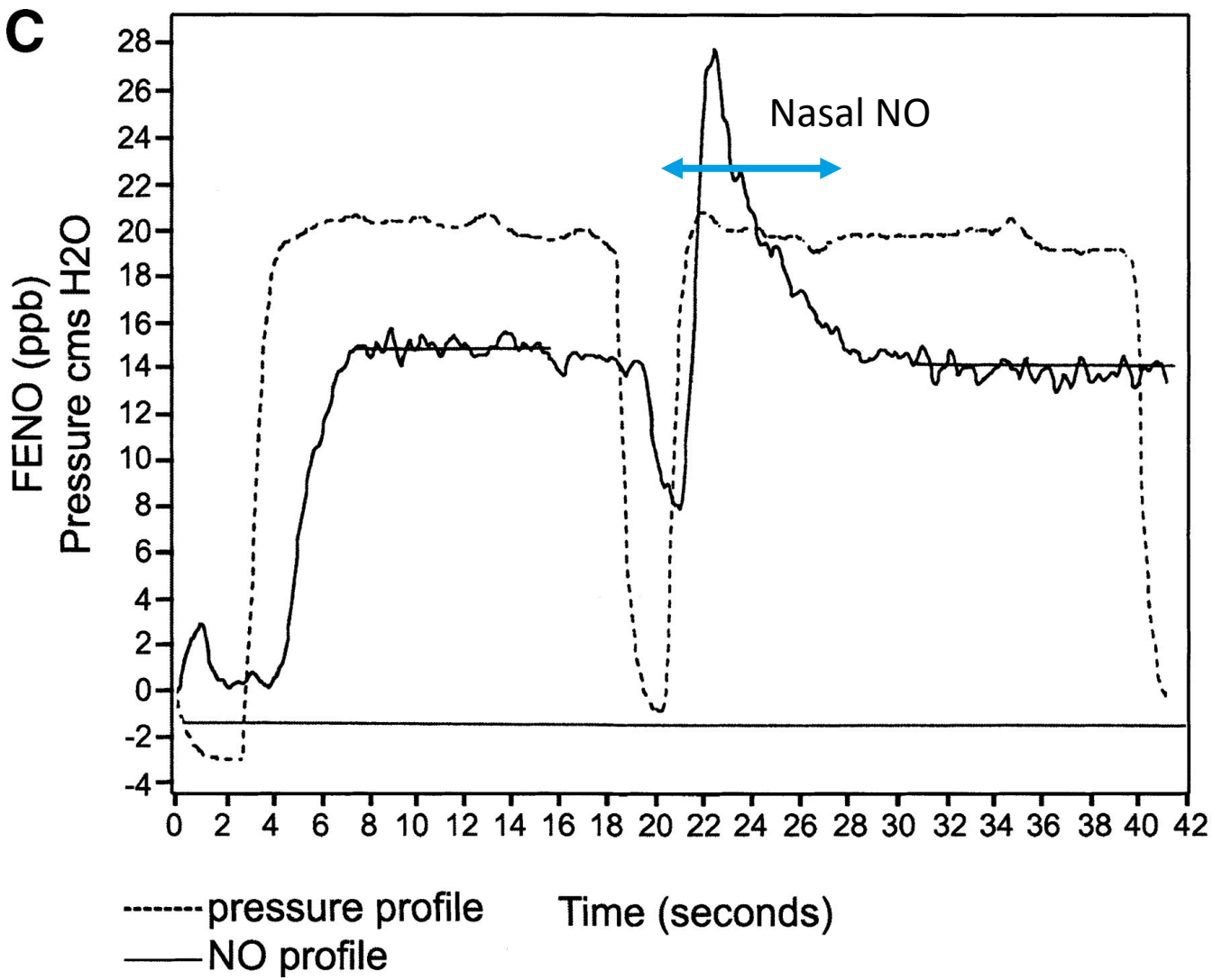


ATS/ERS 2005 recommendation

# 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.

# Is SARS-CoV-2 airborne?

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- Leung *et al*. 2020, 30-40% +ve by fraction, n=17.
- Ryan *et al* 2020: 66-93% +ve R-Tube, test dependent, n=16
- Sammadar *et al* 2021: 23-84% masks +ve days 1-4, n=44.
- Zhou *et al*. 2021, Ma *et al*. 2020: 22-27%, n=9, 52, late in clinical stage
- Feng *et al*. 2021, n=21, all -ve, high dilution.

# Is SARS-CoV-2 airborne?

Northumbria University NEWCASTLE

- 0-93% positivity across studies
  - Saliva is a known source of SARS-CoV-2
  - Most devices cannot exclude salivary / large droplets / fomites.

**Dissolvable strips inside mask- fomite And handling contamination risk**

# Is SARS-CoV-2 airborne?

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- 0-93% positivity across studies
  - Saliva is a known source of SARS-CoV-2
  - Most devices cannot exclude salivary / large droplets / fomites.


COVID-19 positive but **saliva in straw and box**

Zhou L. *et al. J. Aerosol. Sci.* 2021; Ma J. *et al. Clin. Infect. Dis.* 2021

# Is SARS-CoV-2 airborne?

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- 0-93% positivity across studies
  - Virus detected in fine aerosols (<5 um)
  - Levels increase with speech < coughing<< singing
  - Cannot exclude fomites / airflow change effects at cone end.

COVID-19 positive but **air/skin follicle contamination**

Coleman K. *et al. Clin. Infect. Dis.* 2021; Adenaiye O.O. *et al. Clin. Infect. Dis.* 2021.

# Is SARS-CoV-2 airborne?

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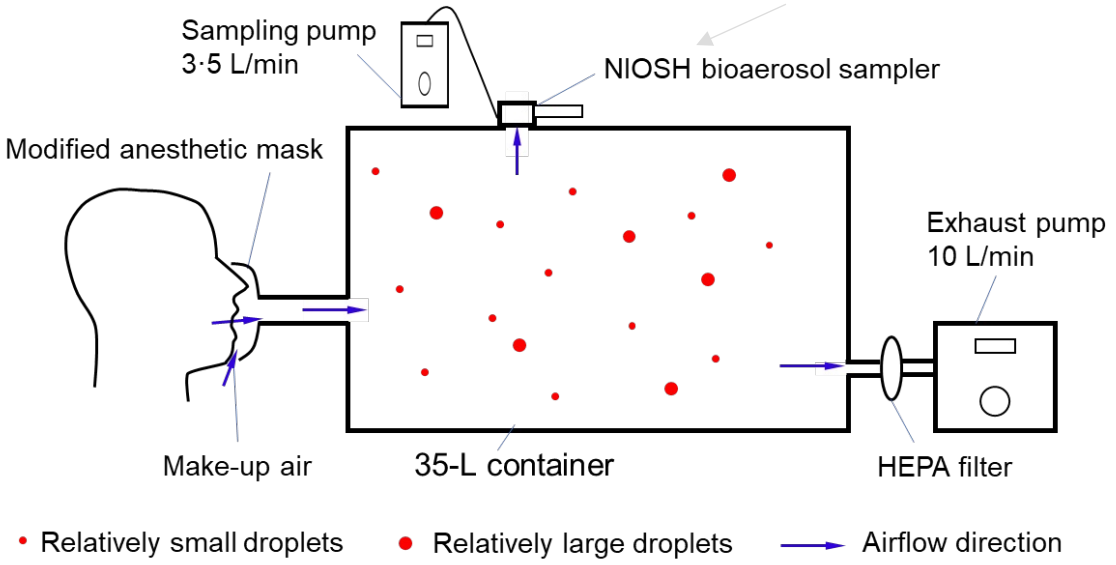
- 0-93% positivity across studies
  - Adequate particle separation.
  - No virus in fine aerosols
  - Could be sub-sampling false negative

**NO VIRUS IN FINE AEROSOL.**




35 Liter container

Est. 26% of resulting air mix adequately sampled.

Feng B *et al. J. Hazard. Mater* 2021

# Is SARS-CoV-2 airborne?

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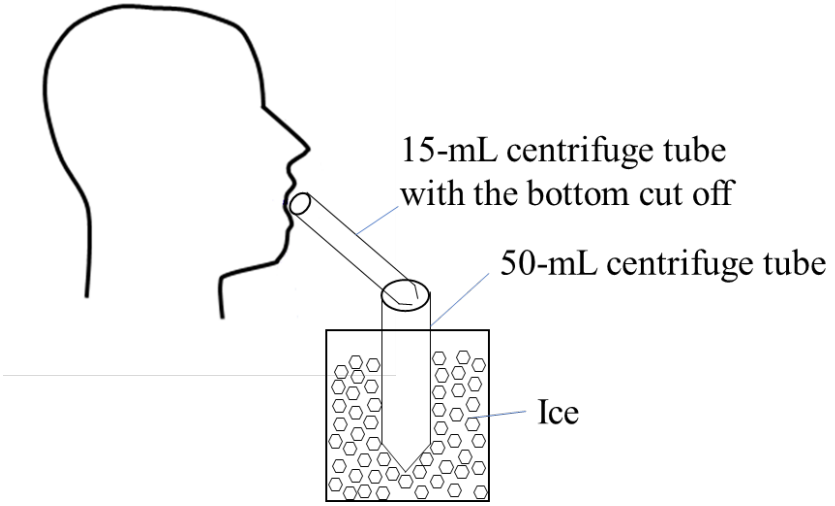
- 0-93% positivity across studies
  - Adequate particle separation.
  - No virus in fine aerosols
  - Could be sub-sampling false negative
  - Same patients 'positive' in traditional EBC sample (saliva)




**Some positive samples in this device from same study as previous slide**

**Main tube can be contaminated w/ drool**

Feng B *et al. J. Hazard. Mater* 2021

# PBM-HALE™: an alveolar condensate collector

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WO2017153755A1: exhaled breath collector – granted UK, USA, JP; WO2019053423A1: cascade impactor array – granted UK, EU

Pictures used with permission

# PBM-HALE™: the platform collects

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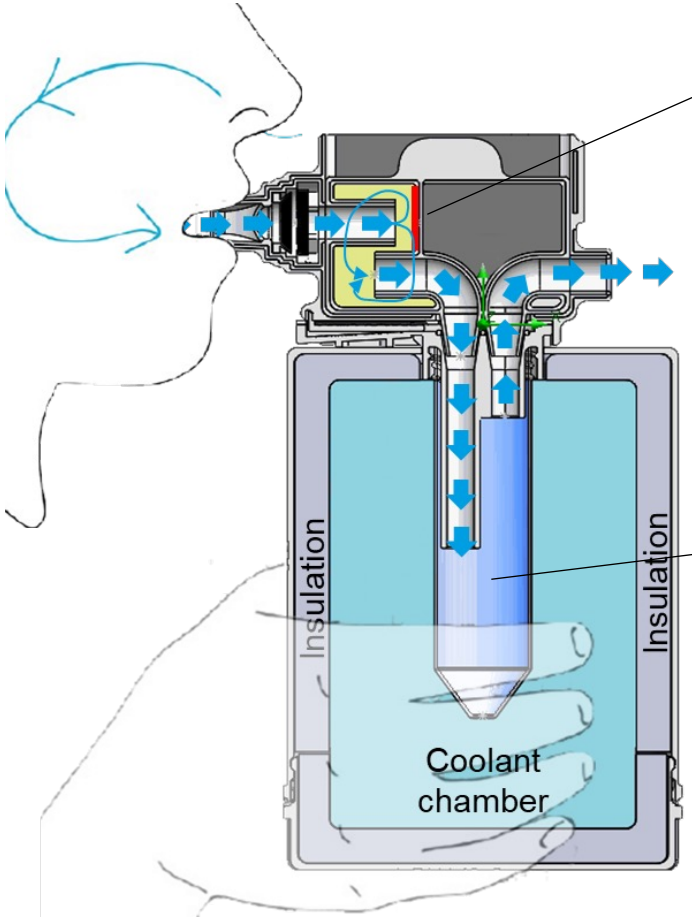
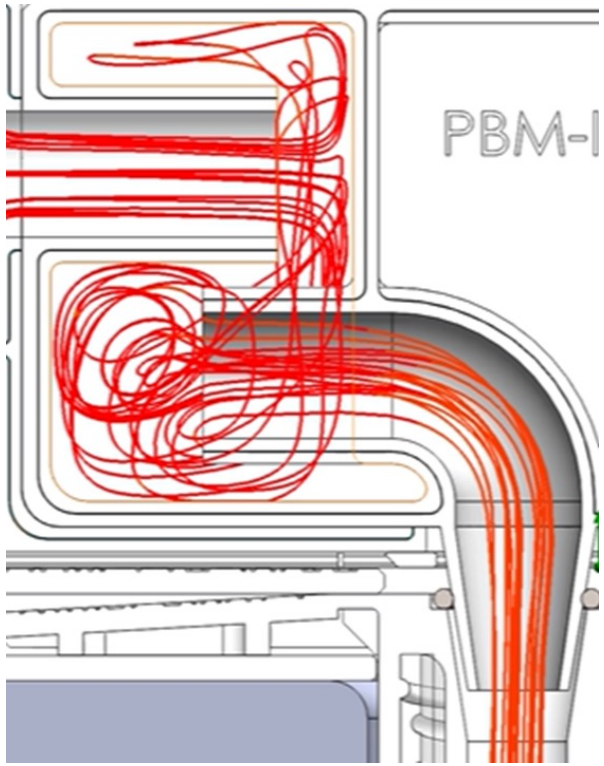



## Large droplets

- By inertial impaction

## Fine aerosols

- By cold condensation




Data by Mr Saqib Ali, Dr Madeleine Combrink

# Inhalation phase FA cooling

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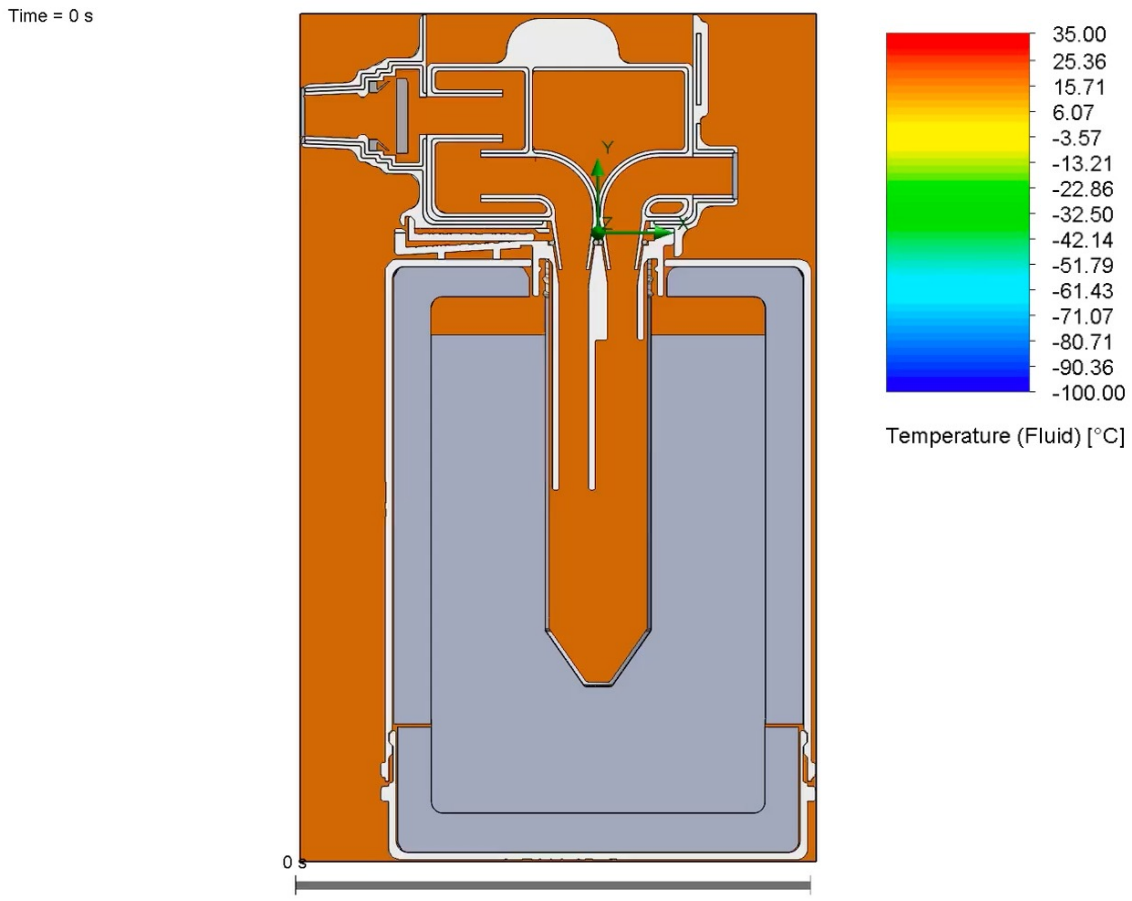
- 5 sec “stabilization”
- Tidal exhalation (0.5L/3 sec)
- No inhalation via the device
- Terminal 48mL of exhalation condensed




5 sec breathing cycle; 10,000 iteration convergence

Data by Mr Saqib Ali, Dr Madeleine Combrink

# Inhalation phase FA cooling

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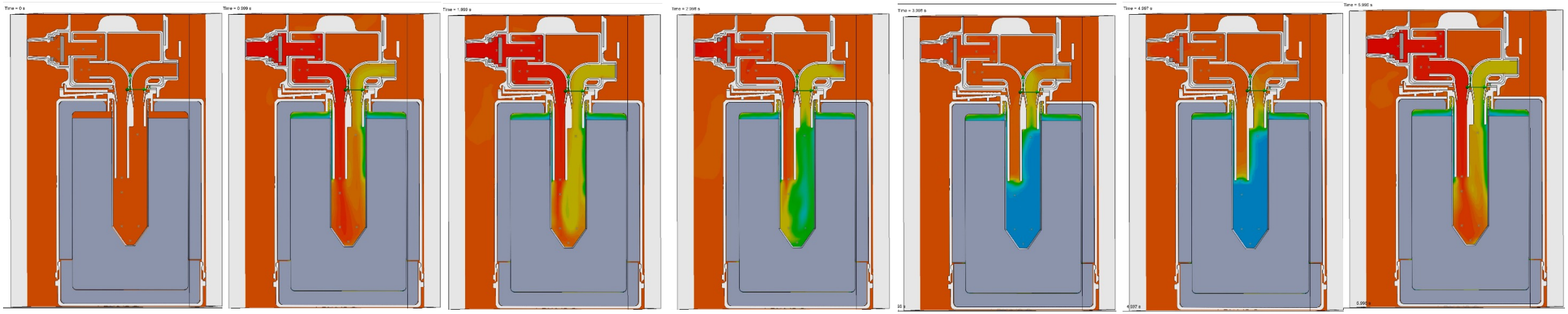
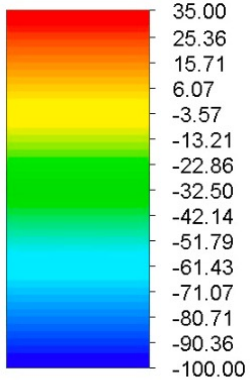
**Exhalation cycle timepoint (sec)**

0 1 2 3 4 5 6



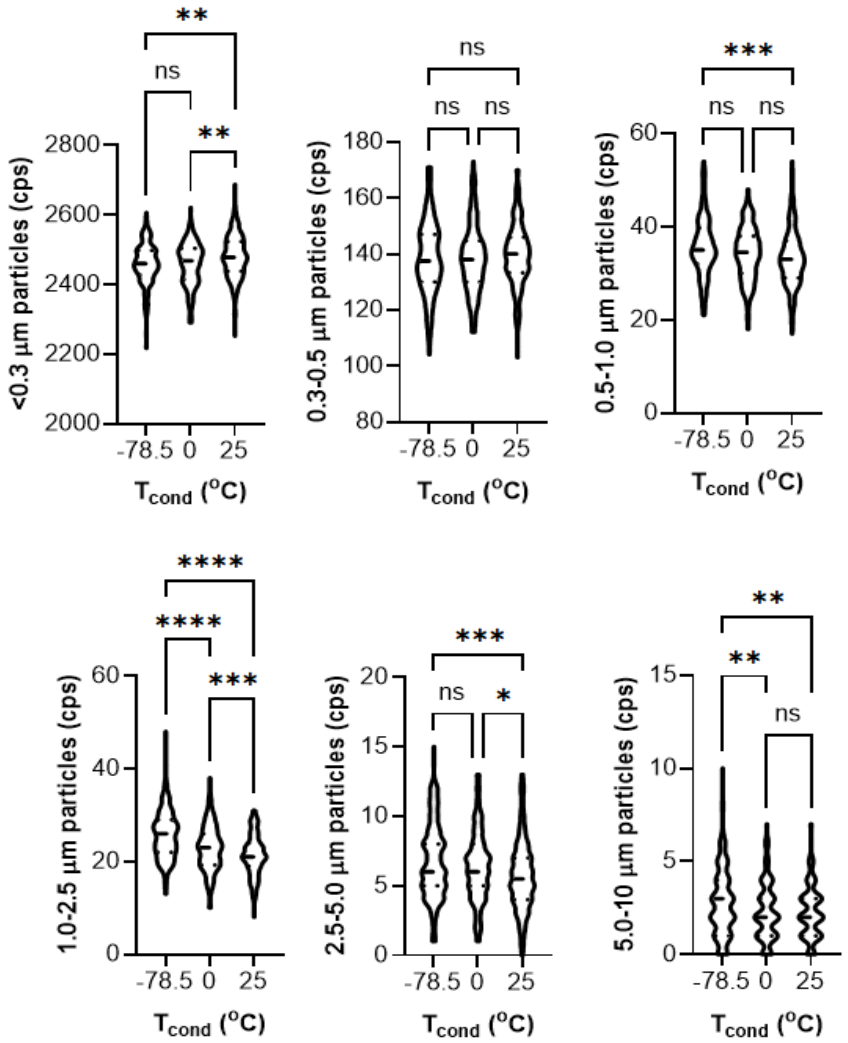



PBM-HALE™ captures
48 ml of FA
from the terminal 83 ml of exhalation

5 sec breathing cycle; 10,000 iteration convergence

Data by Mr Saqib Ali, Dr Madeleine Combrink

# Exhaled particles swell due to condensation




- <5 um particle count drops
- >1 um particle count increases
- Effect maximal at -80°C

- PBM-HALE swells particles to alter aerodynamic properties enhancing capture.

One way ANOVA w/ Holm-Šídák Multiple Comparisons

Data by Mr Saqib Ali, Dr Theodora Mantso

# Fine aerosol capture performance

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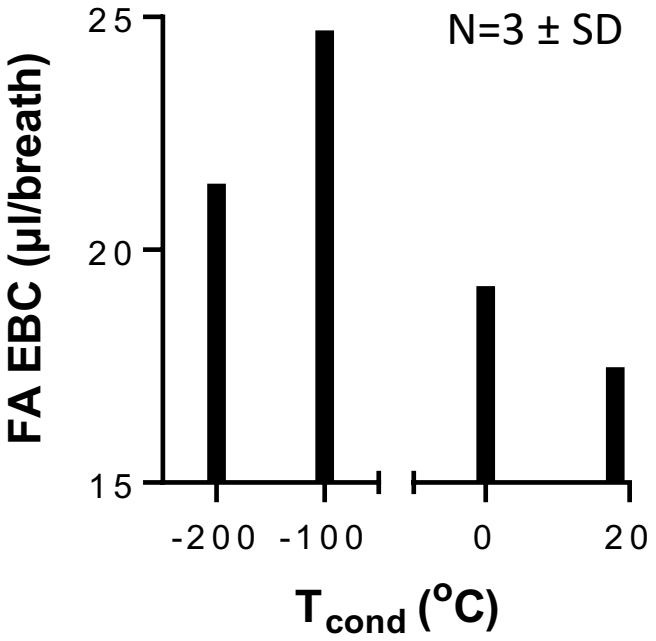
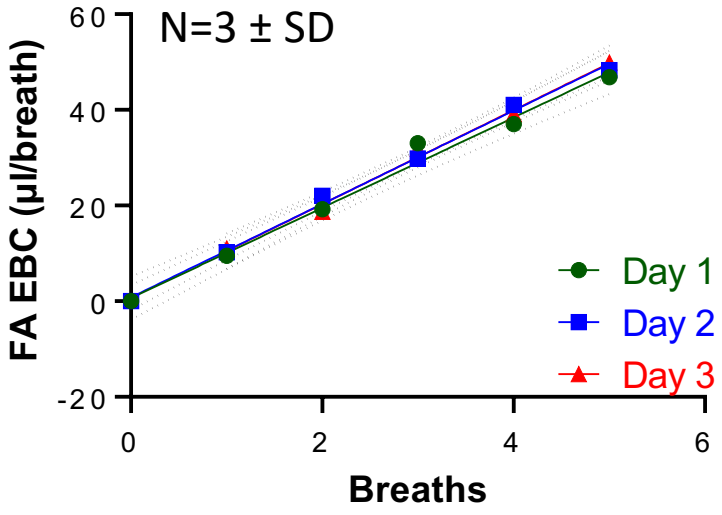
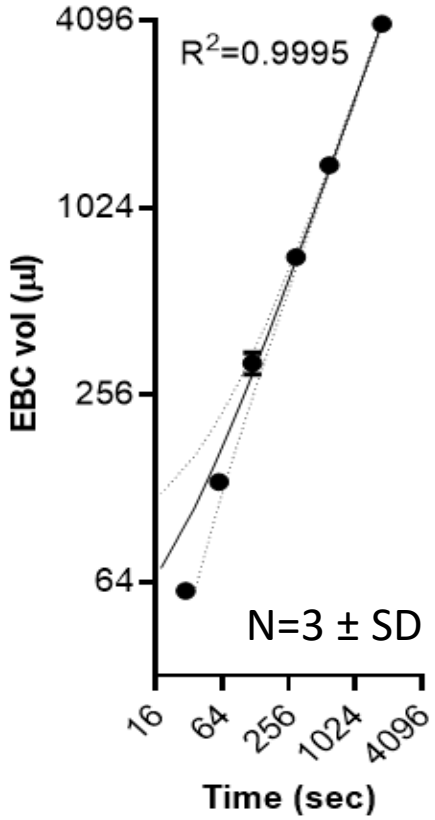
## Condensation Temperature




## Day to day variability




## Sampling duration




WO2017153755A1: exhaled breath collector – granted; WO2019053423A1: cascade impactor array – granted

# Viable bacteria in FA EBC

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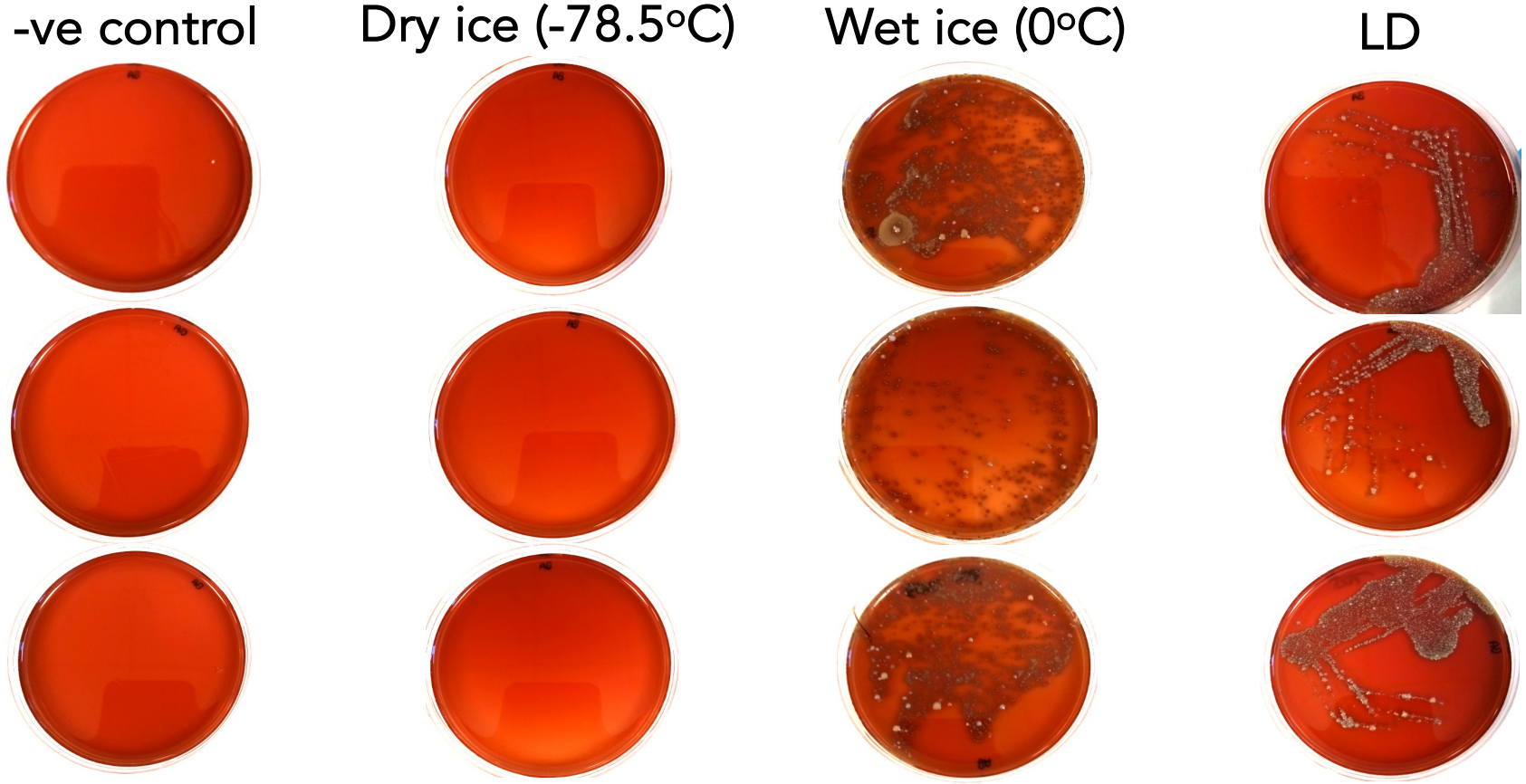
FA Condensation Temperature




2 min sampling period

n = 5, blood agar

Data by Dr John Henderson

# FA and LD Microbiota are distinct

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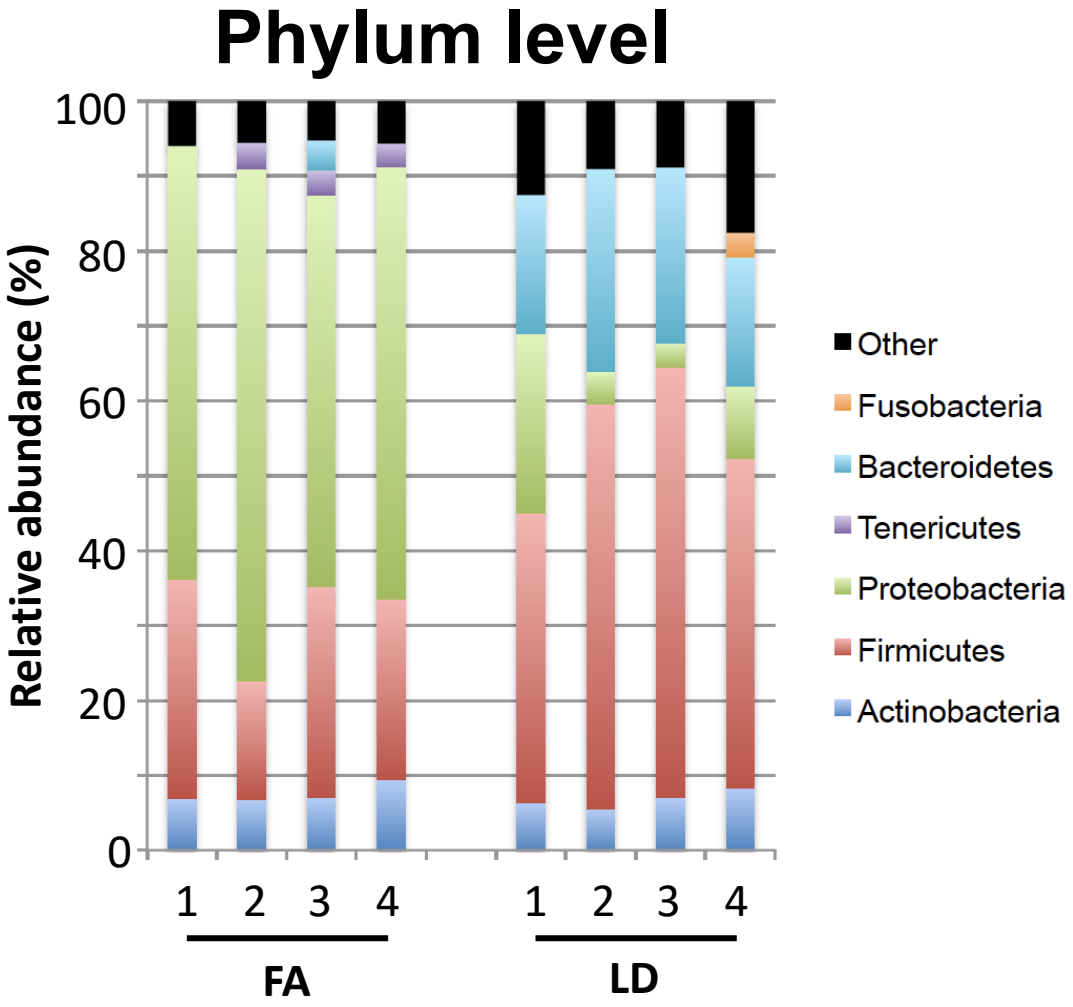


**Invasive sampling**
dominated by
**firmicutes, bacteroides.**

**60% proteobacteria**
reported only in
**healthy lung resection**

(Sze MA *et al. Am. J. Resp. Crit. Care Med.* 2012)

Day-to-day sampling variation, healthy adult male

Data by Dr Louise Usher

# FA and LD Microbiota are distinct

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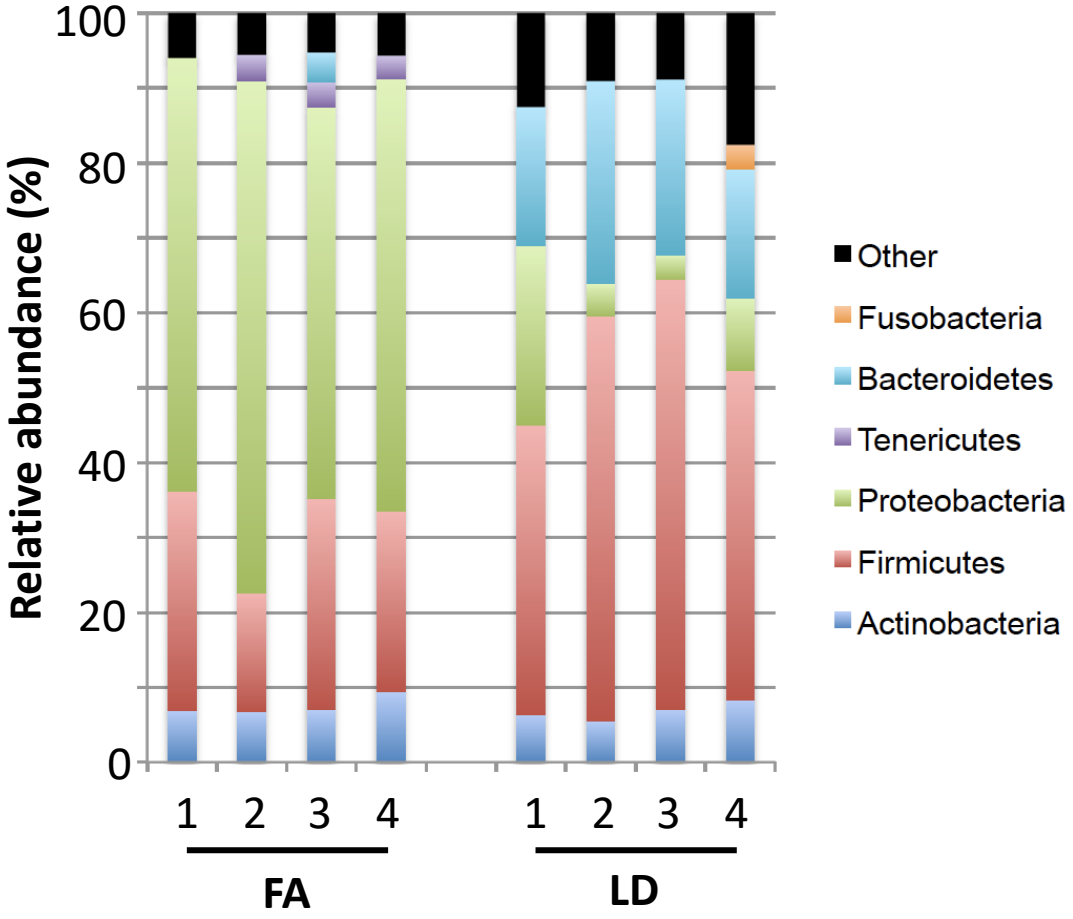
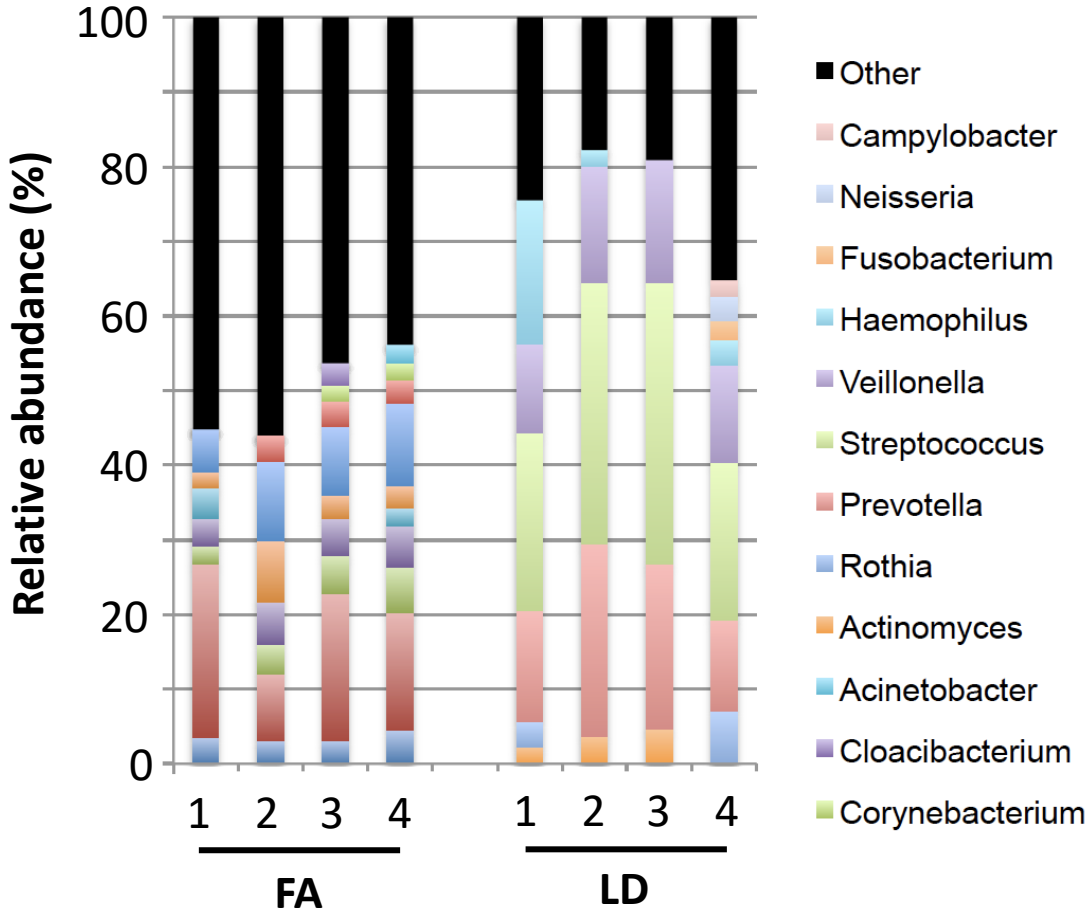
## Phylum level




## Genus level




V3 rRNA gene primers, 40 cycles; Kappa plant 3G polymerase; Qiime analysis using RDP

Data by Dr Louise Usher

# No FA EBC salivary amylase contamination

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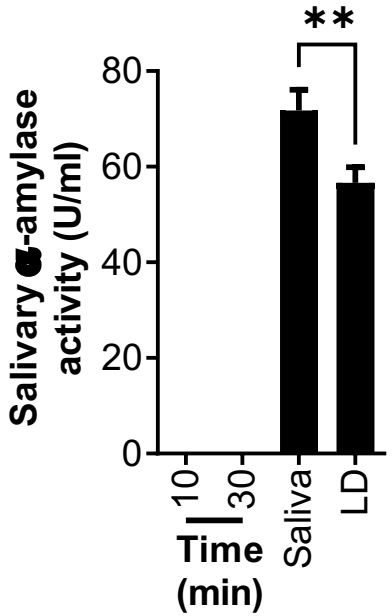


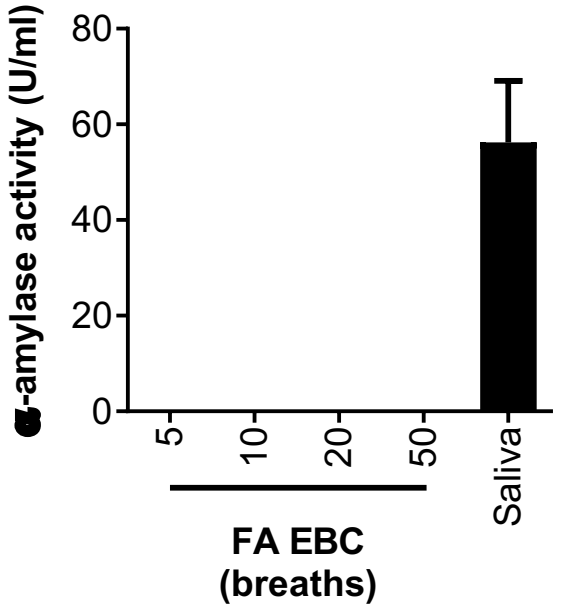


Saliva enzyme levels at least 1,750x less in EBC than in saliva (drool) or device saliva trap even after 30 min.

n = 5.

Data by Miss Zoe Hewitson, Mr Kavit Shah, Dr Theodora Mantso, Dr John Henderson, Prof. M Teixeira group

# Metabolomics of EBC FA

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| Compound | RMM (g/mol) | RT [min] | Relative ion abundance |
|---|---|---|---|
| 1-hexadecyl-glycero-3-phosphate | 396.3 | 1.002 | 810,094 |
| monoacylglyceride | 352.3 | 1.02 | 281,866 |
| LysoPA | 410.2 | 1.032 | 968,316 |
| Palmitoleoylethanolamde | 297.3 | 1.047 | 187,282 |
| eicosatetraenoate | 335.2 | 1.054 | 348,544 |
| Linoleamide | 279.3 | 1.061 | 216,809 |
| Cuscohygrine | 224.2 | 1.067 | 723,759 |
| N-Decanoylglycine | 229.2 | 1.156 | 2,612,124 |
| N-Nonanoylglycine | 215.2 | 1.198 | 1,942,872 |
| cis-3-Hexenyl b-primeveroside | 394.2 | 1.221 | 160,089 |
| N-Lauroylglycine | 257.2 | 1.923 | 286,977 |
| N-Undecanoylglycine | 243.2 | 2.072 | 227,826 |
| phosphatidylethanolamine | 837.5 | 2.388 | 381,518 |
| Gambogic acid | 628.3 | 2.536 | 416,778 |
| 2-Hexenoylcarnitine | 257.2 | 3.062 | 994,821 |
| L-argininium | 175.1 | 3.367 | 502,141 |
| N-Acetylputrescine | 130.1 | 3.519 | 192,382 |

Compounds detected by MS1:

- C6-C24 fatty acids.
- Phospholipids & precursors.
- Glycans.
- Medications.
- Drugs of abuse.
- Dietary compounds.

*Additionally:*

- *20 multiple HDBM hits.*
- *104 novel compounds.*

Data generated by Dr John Henderson at the Northumbria University Metabolomics Core Service

# Aerosolised SARS-CoV-2 VLP Capture

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PariBoy Classic nebuliser:

- Mean droplet diameter 3.5 μm
- 67% of mass in < 5 μm
- 5 min sampling

Particle types:

- Polysterene beads (118 nm, -71 mV, di$H_2O$)
- Neutral liposomes (168 nm, -20 mV, PBS)
- Negative liposomes (188 nm, -72 mV, PBS)
- Lentiviral VLP (MLV; 193 nm, -31 mV, PBS)
- SARS-CoV-2 VLPs (155 nm, -17 V, PBS)

Rudiger Gross, Janis Mueller, Jan Munch
U. Ulm

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- Negative liposomes (188 nm, -72 mV, PBS)
- Lentiviral VLP (MLV; 193 nm, -31 mV, PBS)
- SARS-CoV-2 VLPs[1] (155 nm, -17 V, PBS)

## Sampling conditions:

- 5 ml sample
- 5 min nebulization
- ~1.5 ml of dry ice condensate captured

## Sample analysis:

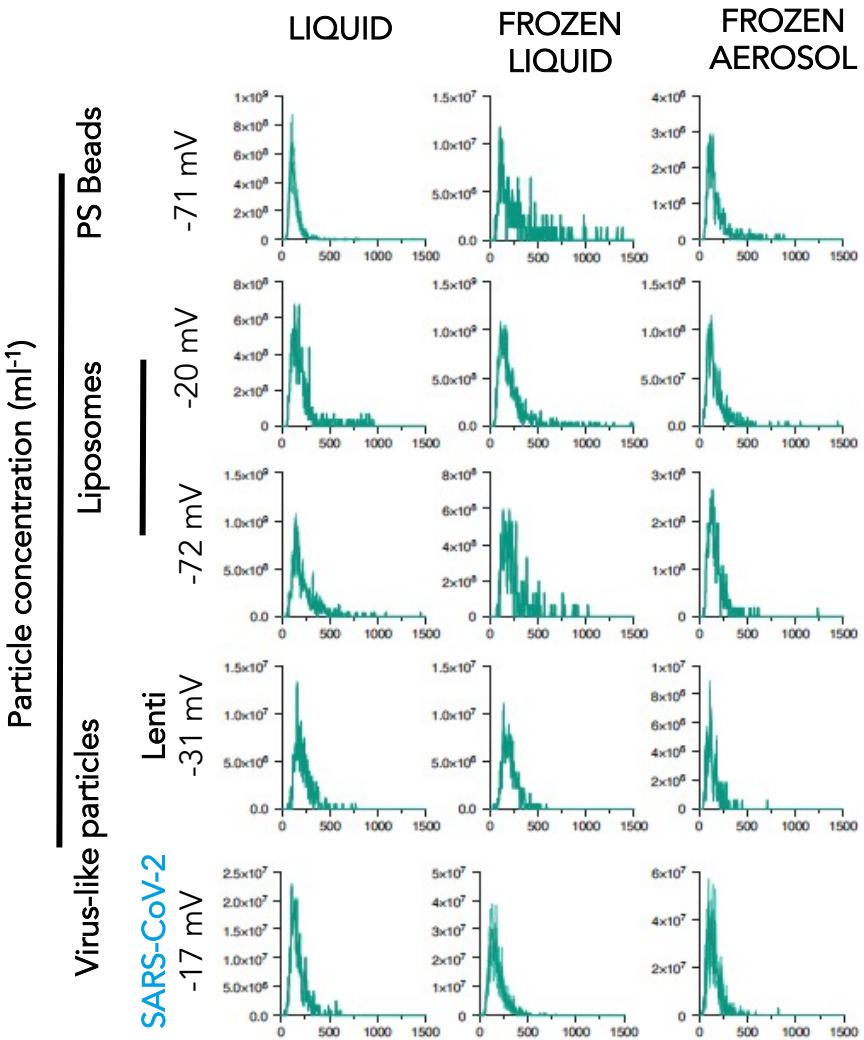
- Particle size and concentration
- Fluorescent Nanoparticle tracking analysis (Malvern; 488 nm FluoSpheres, TopFluor liposomes, YFP, or scatter)
- Unfrozen input, Frozen input, Condensate.

1. Based on fbioe.2020.00862 protocol

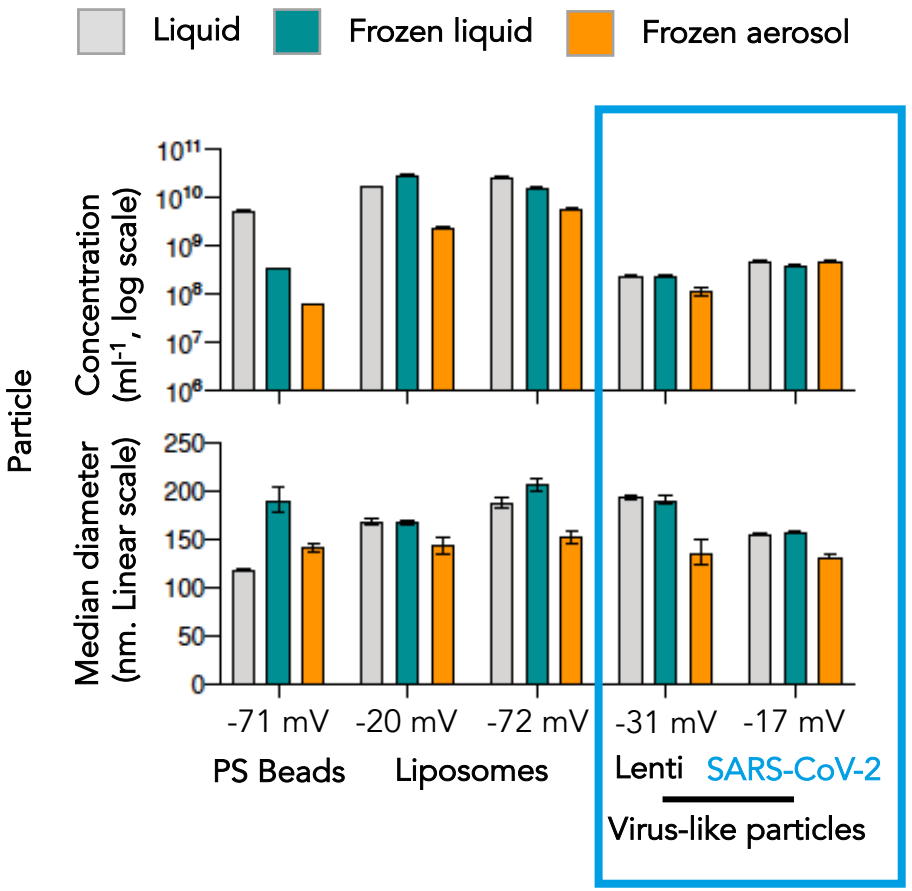
Rudiger Gross, Janis Mueller, Jan Munch
U. Ulm

# Aerosolised SARS-CoV-2 VLP Capture

Northumbria University NEWCASTLE




N=3, Shading covers SEM




## Virually no loss of VLP size or structure

Aggregation with highly charged particles:

- Drop in concentration
- Rise in particle size
- Beads and liposomes

Data from Rudiger Gross, Dr Janis Mueller, Prof Jan Munch
U. Ulm, Germany

# COVID19 Exploratory Pilot Study

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## Inclusion criteria:

- NP swab positive
- Within days 0-5 of symptom onset

## 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:

- 1.5 years to complete
- No SARS-CoV-2 RNA in FA

Dr P. Katsaounou and G. Magiorkins groups

# COVID19 Follow-up Pilot Study

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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
- Saliva
- Fine Aerosol, Large Droplets

Prof. M.M. Teixeira and Dr R.S. Aquiar groups

# SARS-CoV-2 interim clinical data

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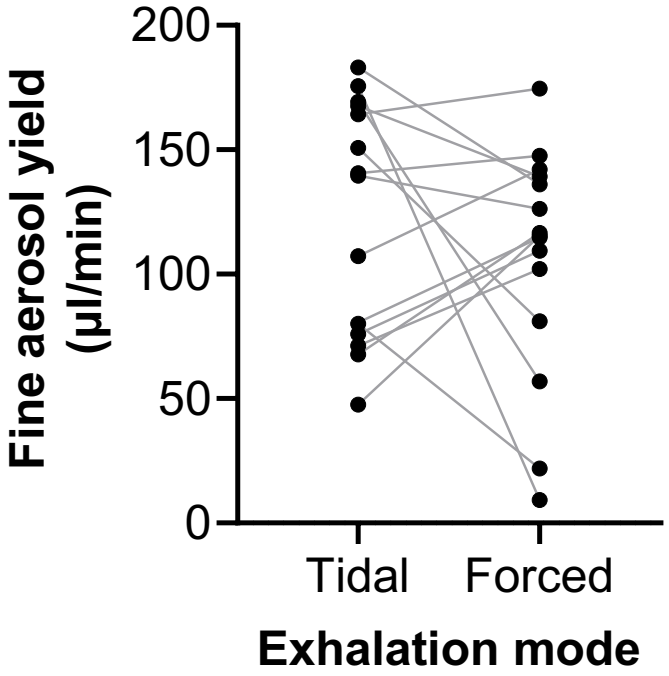
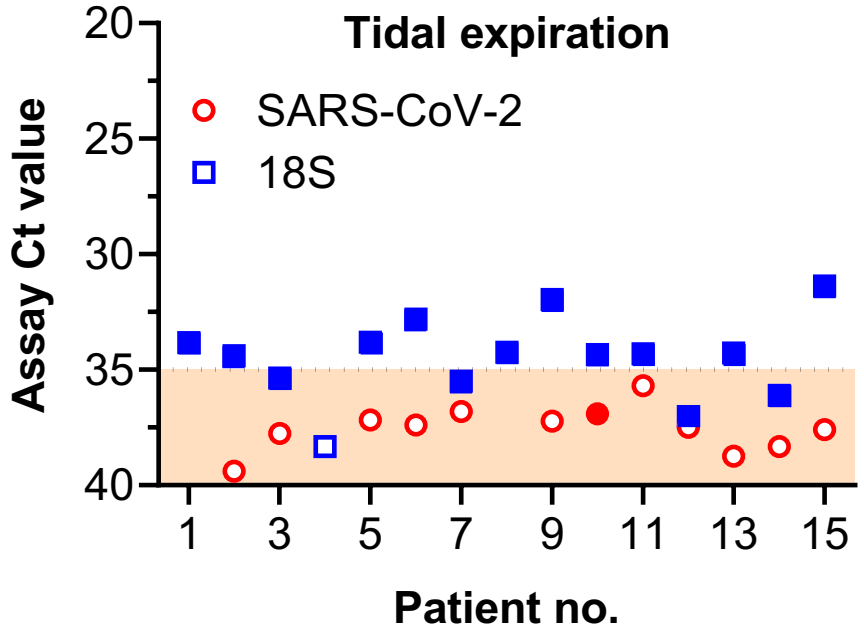
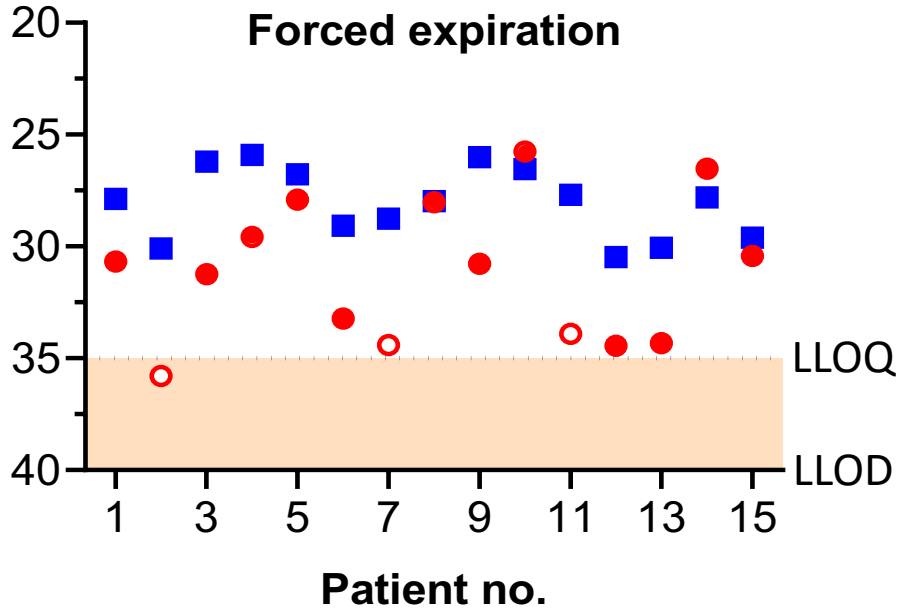
## FA volumes

Tidal vs singing: ~120 ul/min




## Total host RNA and SARS-CoV-2 RNA




100% of singing samples positive, 1 min sample

90x increase in host total RNA yield

8.72x more sample assayed in Brazil study vs Greece study (or 2.95 cycles higher signal).

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)

# FA host RNA not due to saliva contamination

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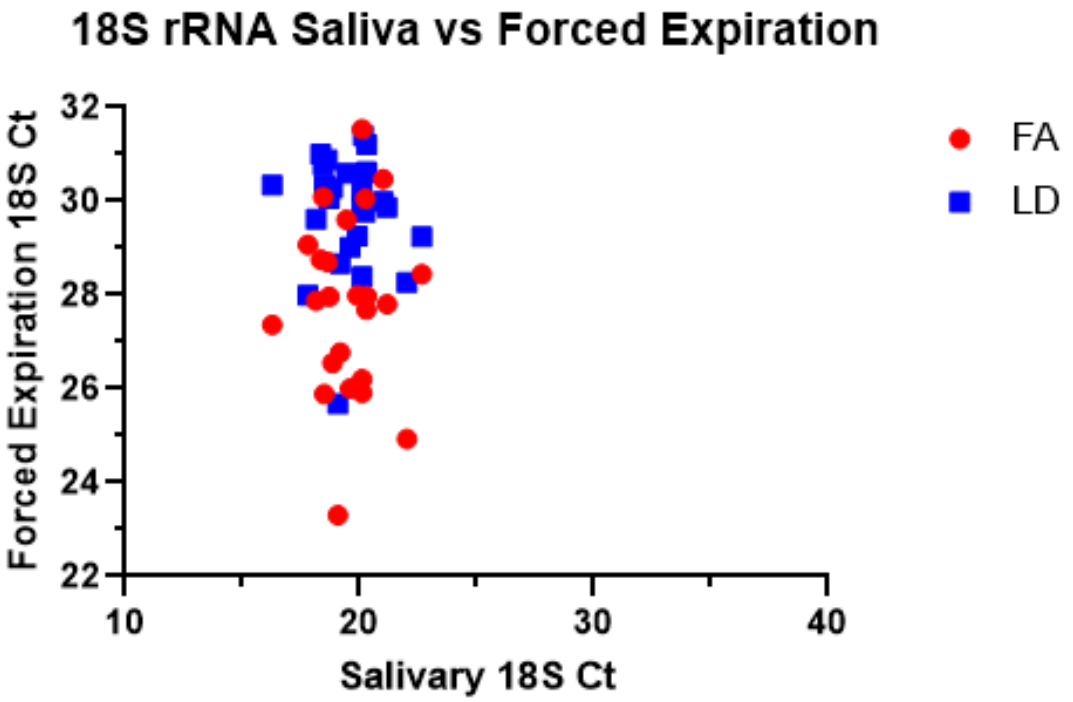


- No 18S rRNA (total RNA) correlation between:
  - Saliva vs LD (p = 0.8718)
  - Saliva vs FA (p = 0.6670)

- FA total RNA is not due to saliva contamination

# Conclusions

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## SARS-CoV-2 in breath:

- Present in saliva
- Not found in tidal alveolar FA, LD
- High FA levels in speech

- **Mode of exhalation affects rate of emission and detection.**

- **More than Vx needed to resolve pandemic**[1]

## PBM-HALE™ Breath Fractions[2]:

- Cell membrane components
- DNA, RNA, protein
- Drugs of abuse
- Bacteria, fungi

- FA not contaminated by:
  - Saliva
  - Ambient aerosols

1. Lazarus J. *et al.* Nature 2022; 2. Henderson J. *et al.* MedRxiv 2022

# Future work

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## Beyond COVID-19

- Total RNA-SEQ
- Host cytokine changes
- Flu, RSV, TB, *Haemophilus*

- Australia & China industry collaborations

- 2 million uses per month

## Asthma & paediatric wheeze

- T2 High vs T2 Low asthma
- 7 subtypes, different cytokines
  - No neutrophilic stratification test available
  - Pharma Rx development
- Bacterial infection AMR

- Infections + asthma typing = Paediatric wheeze

# Acknowledgements


**Moschos lab Northumbria**
Dr John Henderson
Dr Andrew Nelson
Dr William Cheung
Dr Pep Canyelles-Pericas
Mr Craig Clements
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