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COVID-19 Diagnosis through RT-qPCR in Exhaled Breath using a Novel Hand-Held Breath Sampler.

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Founder and Chief Scientific Officer, PulmoBioMed Ltd.

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Oxford Global's NextGen Omics UK 2021

Overview

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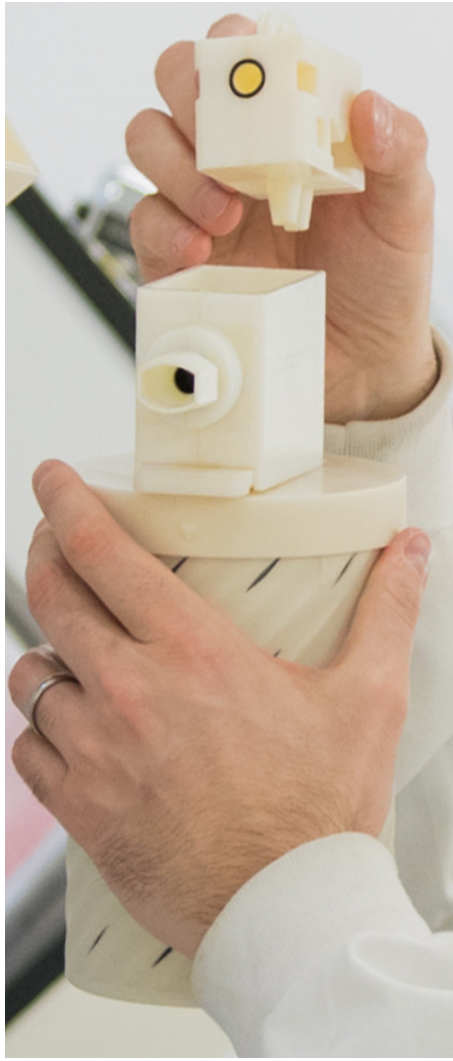
- Exhaled Breath Condensate for Diagnostics

- The PBM-HALE™ approach
  - Fine Aerosols and Large Droplets at source
  - Biomarker discovery
  - Microbial capture

- Clinical progress update.



PBM-HALE™

Diagnosing from Exhaled Breath Condensates (EBC)

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Breath is 95% hydrated:

- Volatile compounds
- Vapour & aerosols.
- Biological molecules.

Health and Disease indicators:

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

Challenges to clinical use

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

RTube™

Poor process control

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™: the device

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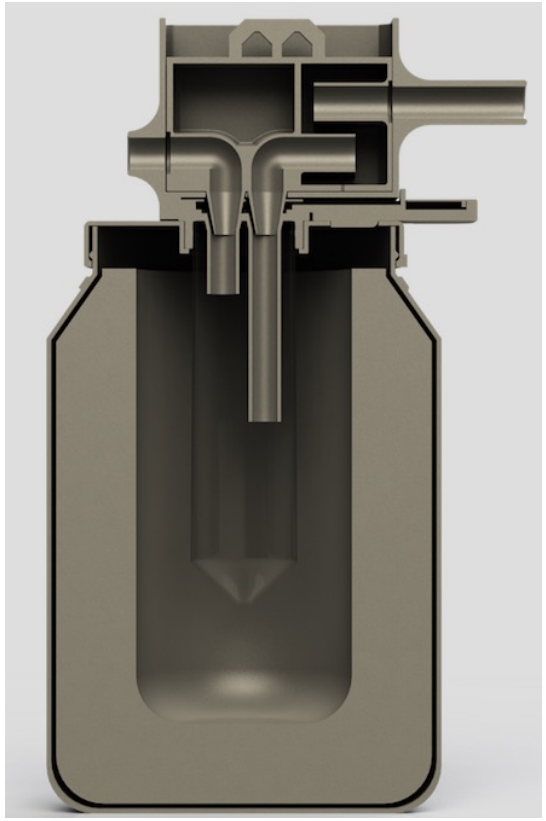

WO2017153755A1: exhaled breath collector – granted; WO2019053423A1: cascade impactor array – granted.

PBM-HALE™: the device

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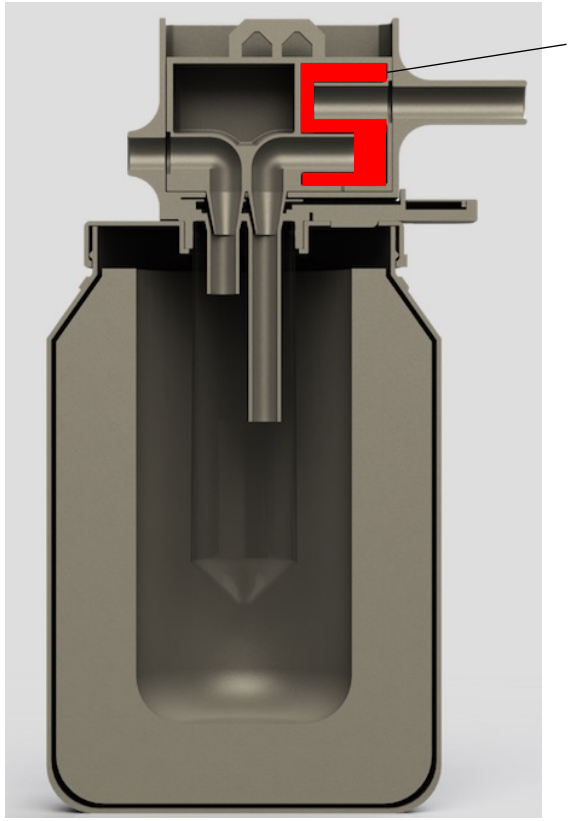

Large droplets (LD)

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PBM-HALE™: the device

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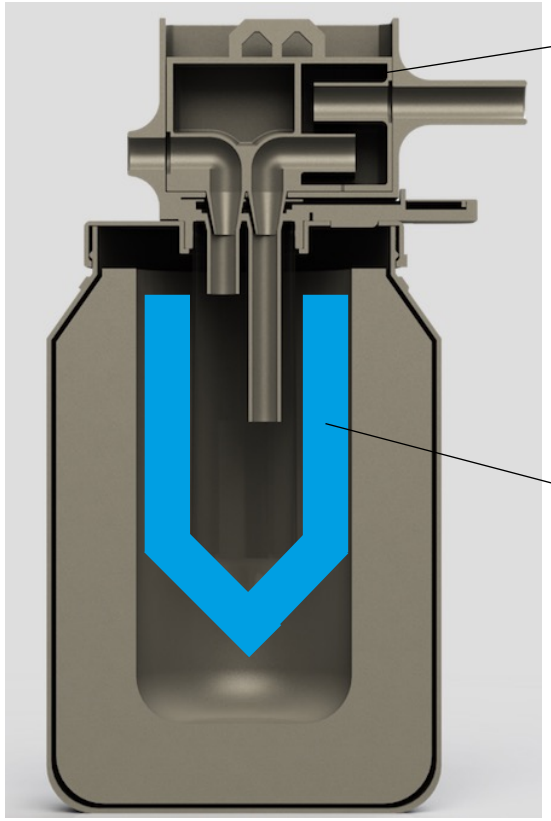

Large droplets (LD)

Fine aerosols (FA)

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

Inhalation phase FA cooling

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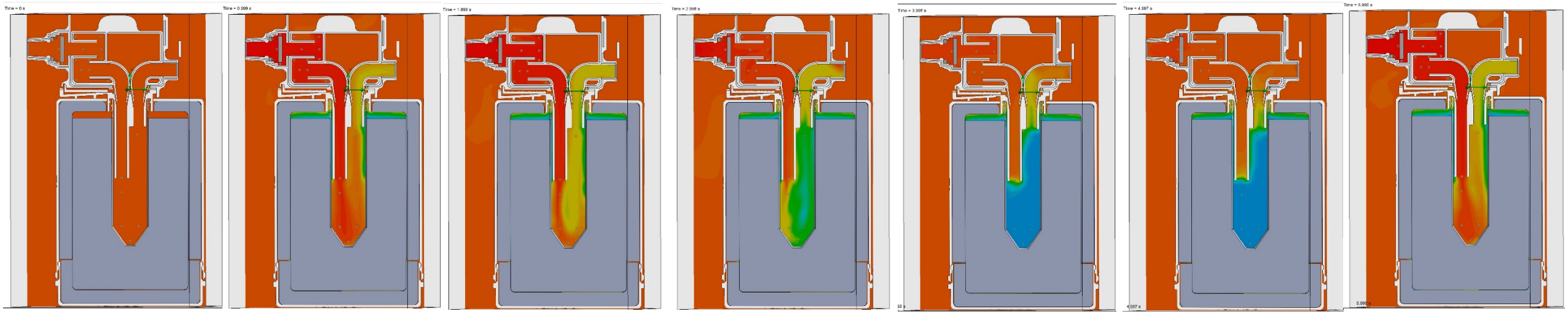
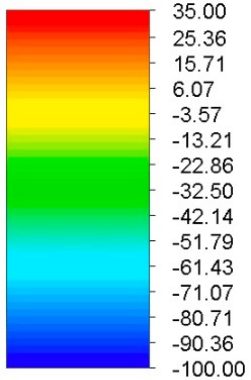
Exhalation cycle timepoint (sec)

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

FA swelling enhances capture 6x

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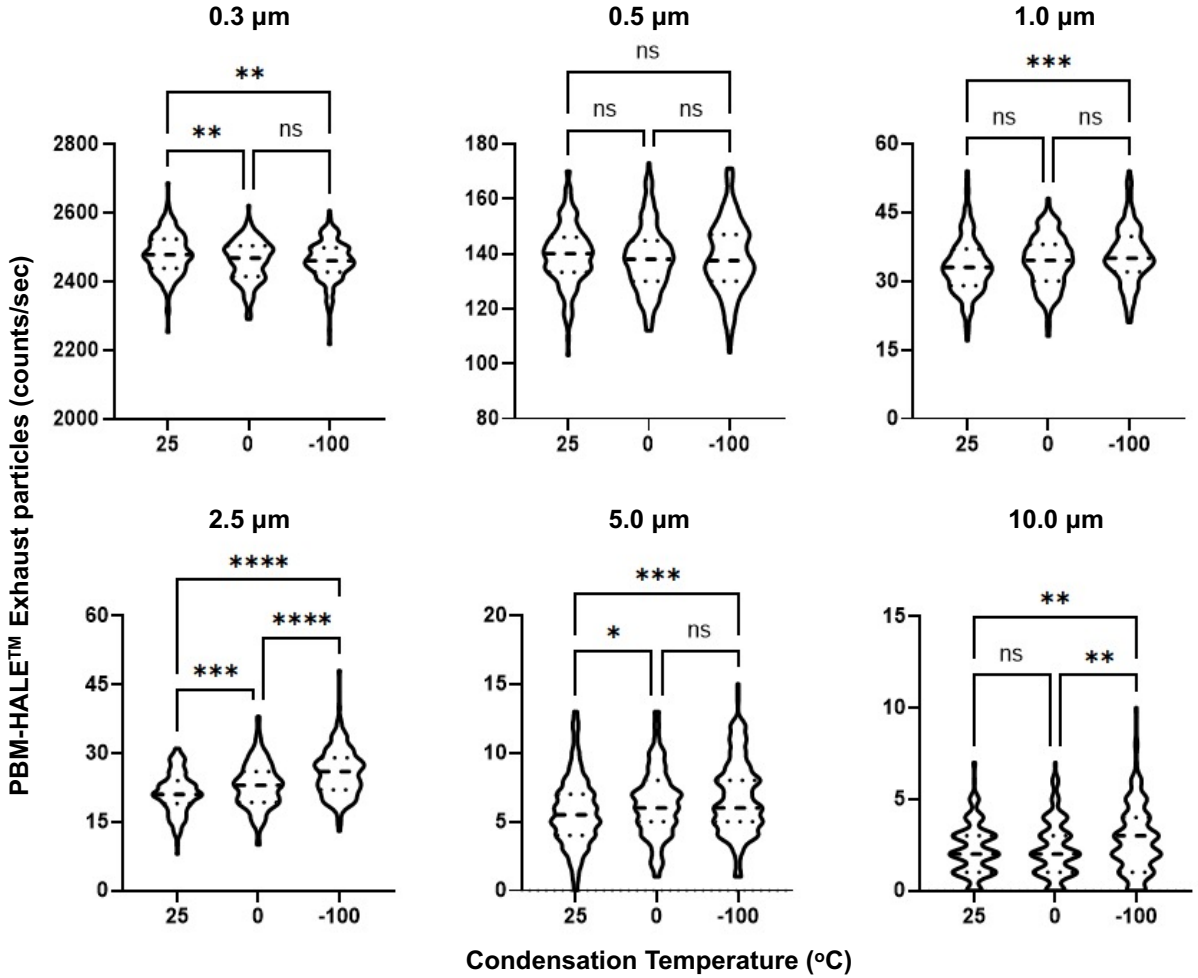
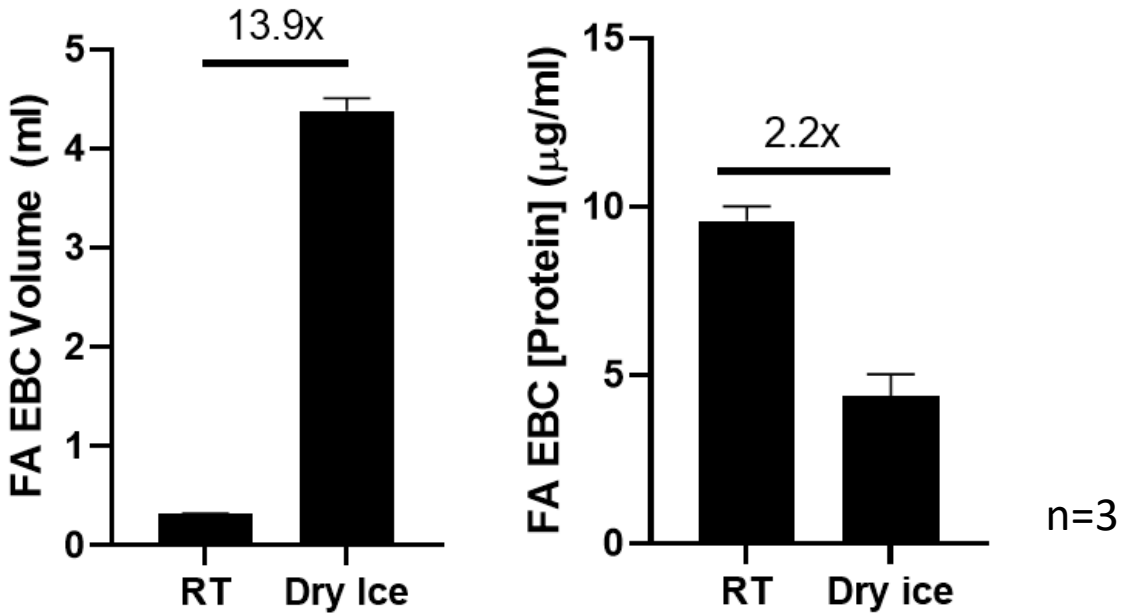
FA Particle Size vs $T_{Condesning}$



Content enrichment



n=3

PBM-HALE™ enhances FA capture by ~6.3x

Data by Dr Theodora Mantso

Performance in FA EBC capture

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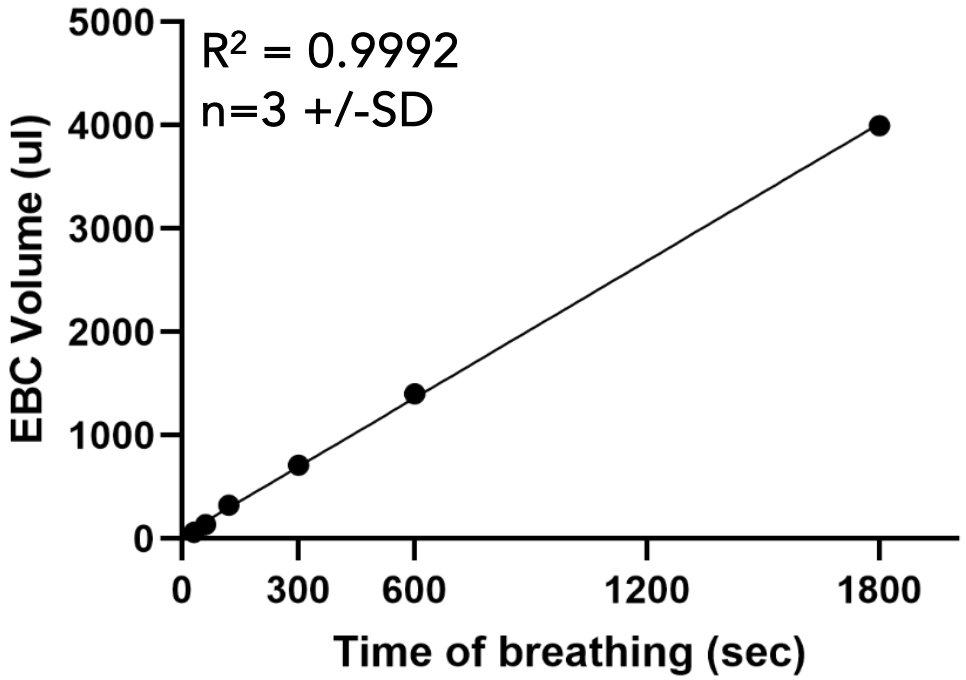
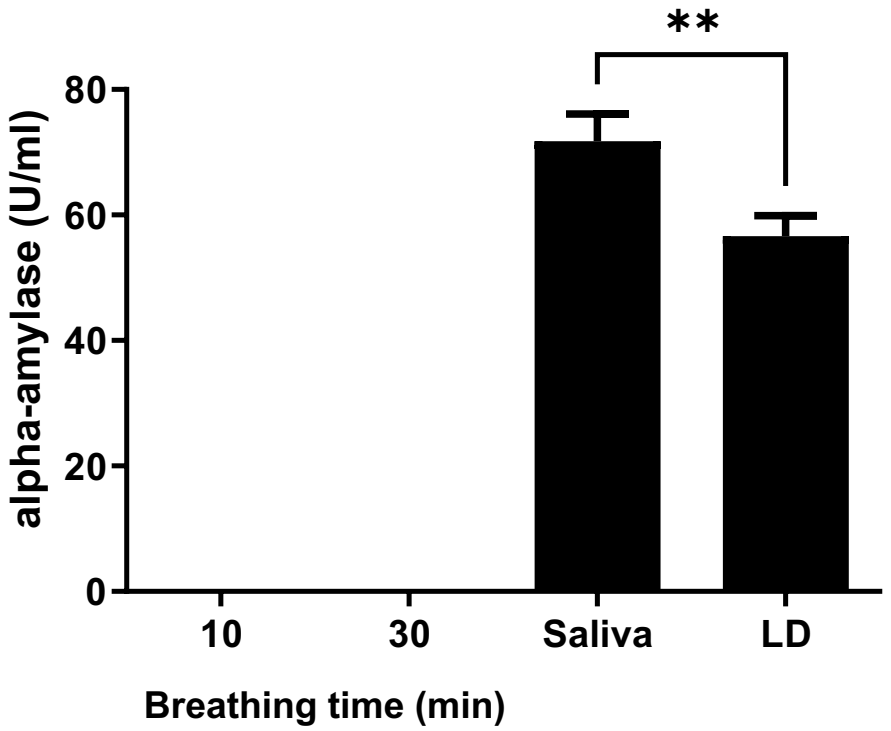
FA Sampling Consistency



R^2 range 0.9980-0.9992

No Salivary Contamination



>1/1750x dilution
>500 samples to date

Data by Dr Theodora Mantso and Dr John Henderson

Consistent [RNA] in FA EBC

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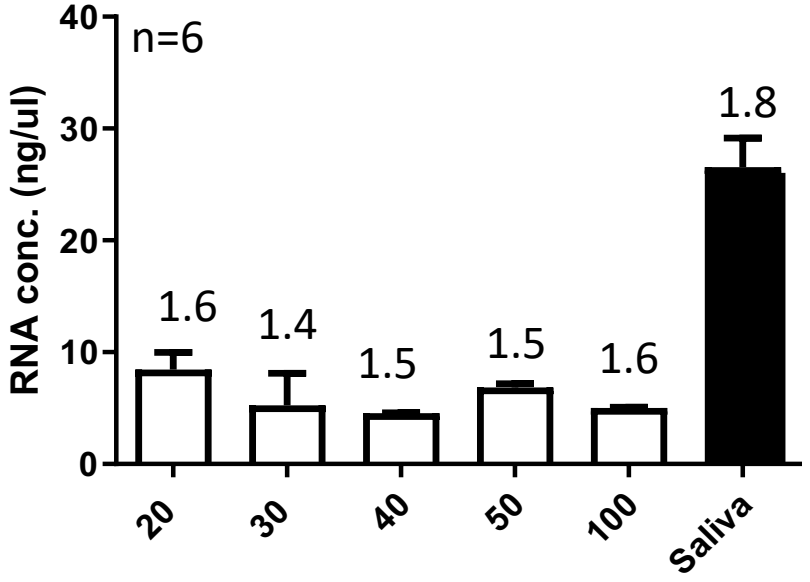
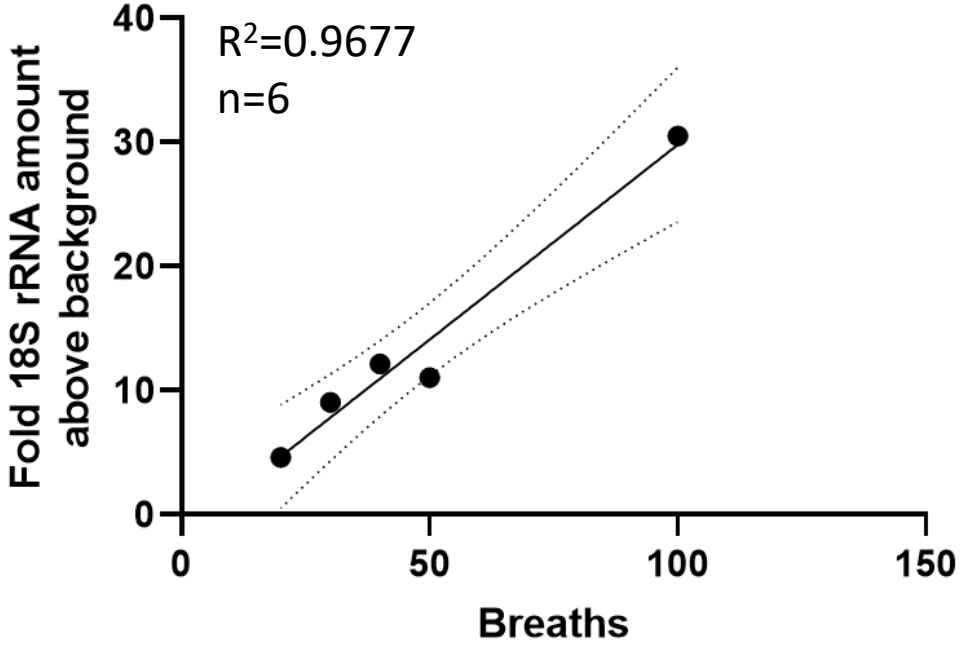
Total RNA concentration



No statistically significant difference in total [RNA].

260/280 ratios reported per column

18S rRNA yield



18S yield increases linearly with time

Data by Dr John Henderson

Metabolomics of EBC FA

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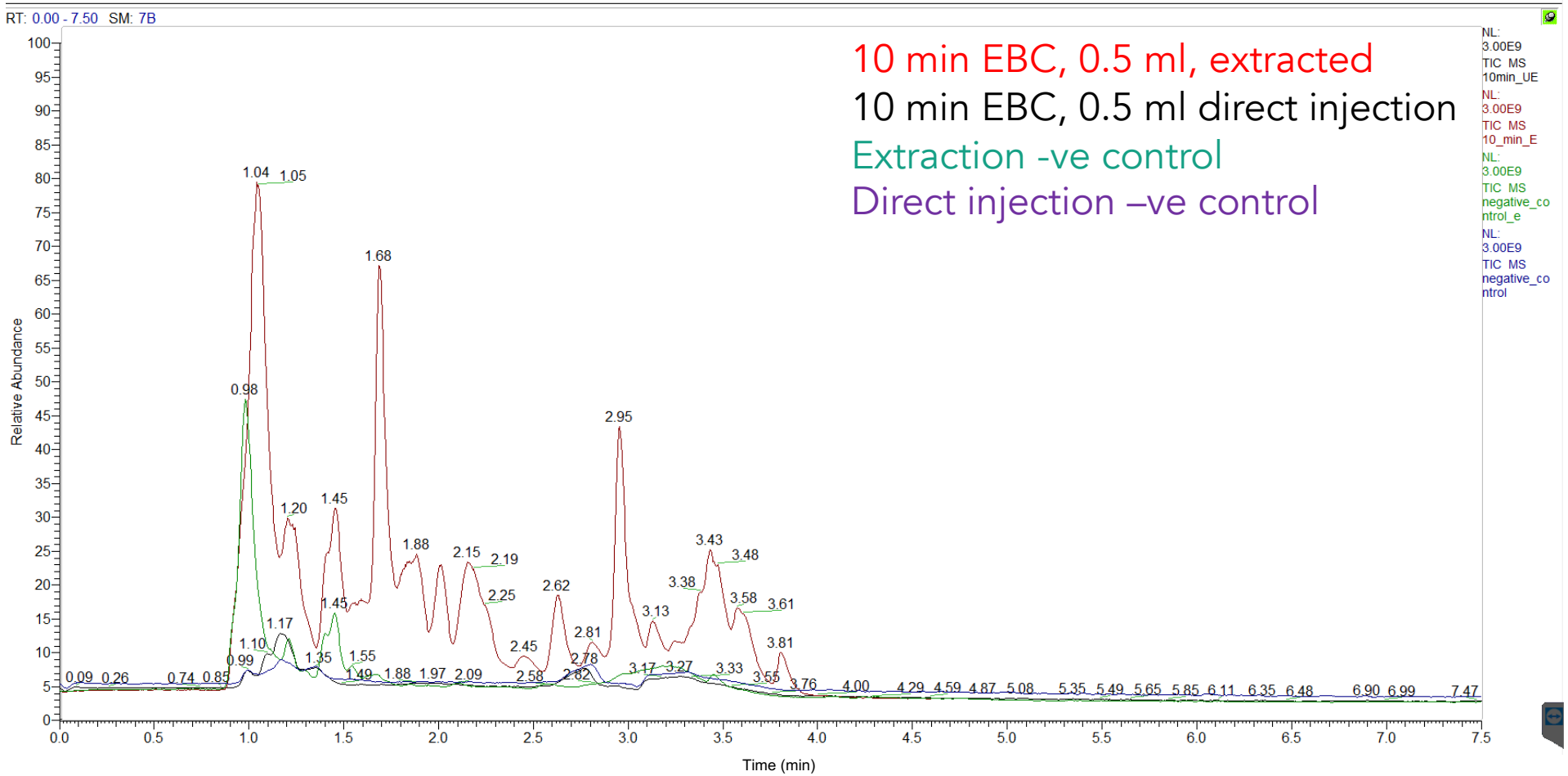

No metabolites detectable without acid extraction

n=5

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

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

Bacteria in EBC FA

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Condensation Temperature

-ve control	Dry ice (-78.5°C)	Wet ice (0°C)	Saliva

2 min sampling period

n = 5, blood agar

Data by Dr John Henderson

FA and LD Microbiomics

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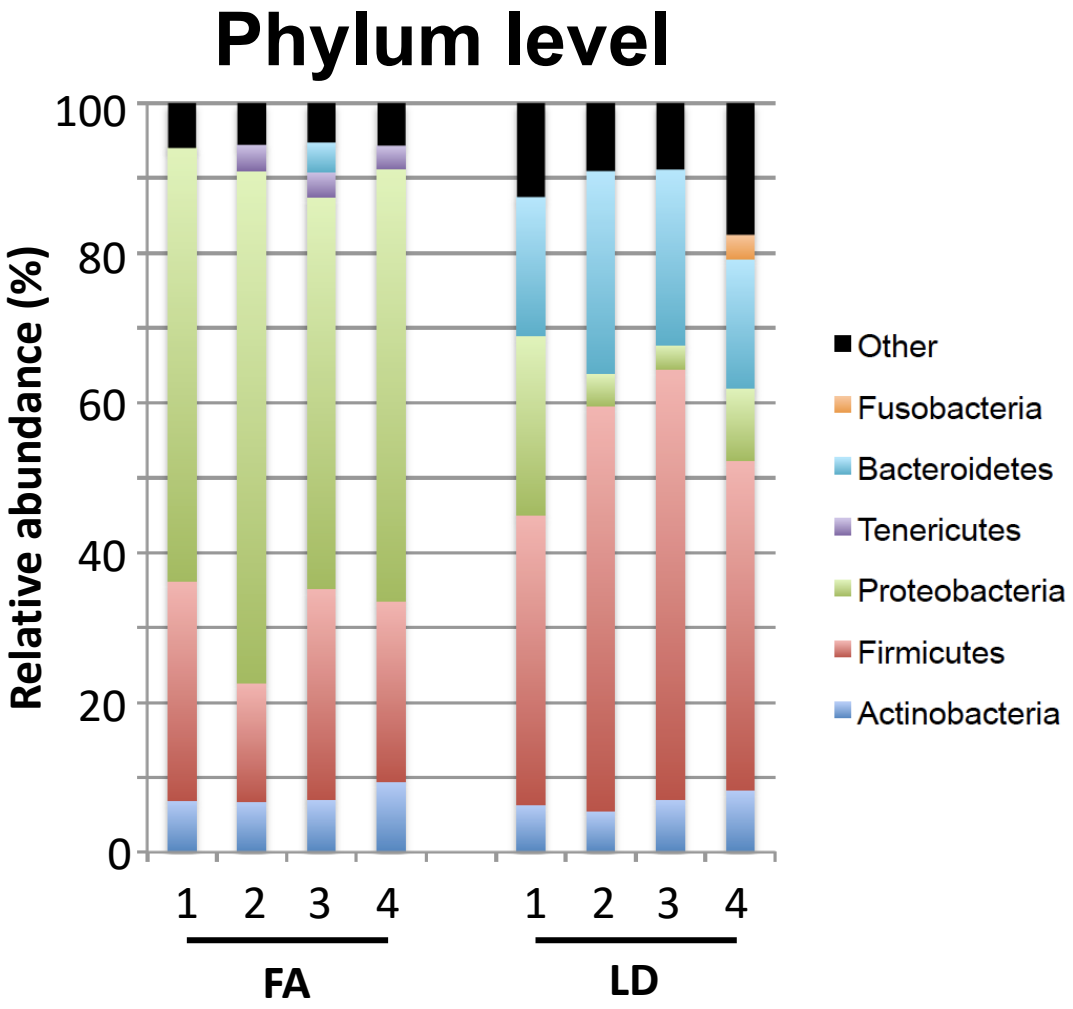

Invasive sampling dominated by firmicutes, bacteroides.

60% proteobacteria reported only by healthy lung resection

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

Data by Dr Louise Usher

Case study: cause of mild cough

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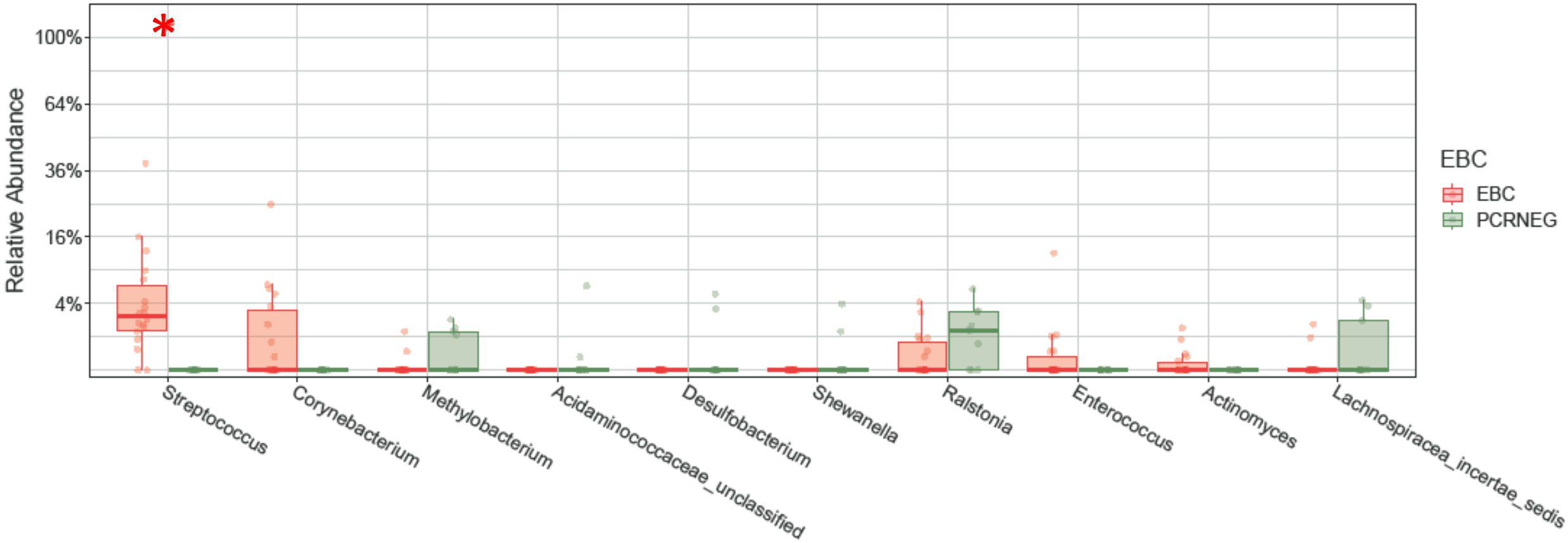

Higher DNA content vs background / kit controls

Detection of *Streptococcus* (FDR q = 0.019) w/out extraction

Data by Dr John Henderson, Dr Andrew Nelson

Aerosolised SARS-CoV-2 VLP Capture

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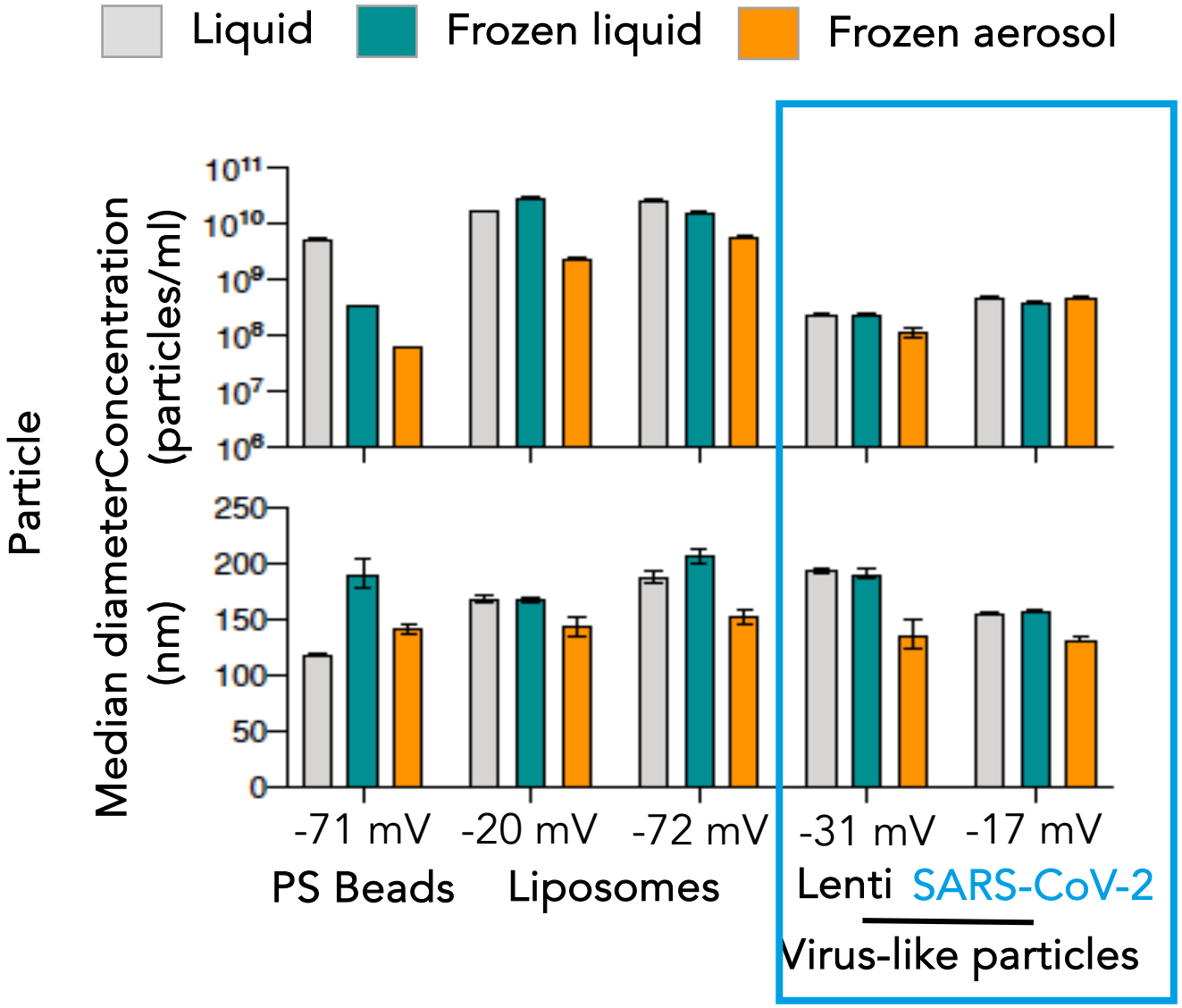

Virtually no loss of VLP size or structure

Aggregation with highly charged particles:

- Drop in concentration
- Rise in particle size

Data from Rüdiger Gross, Dr Janis Müller, Prof Jan Munch U. Ulm, Germany

Aerosolised infectious pseudotyped virus capture

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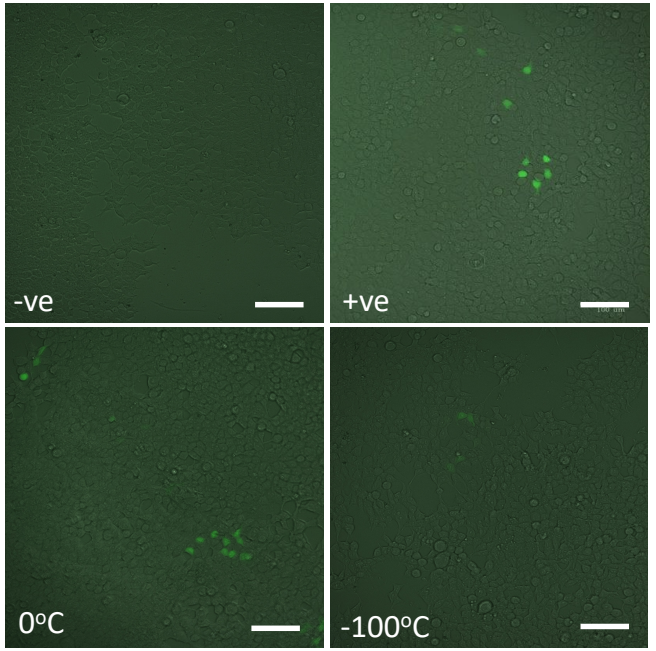

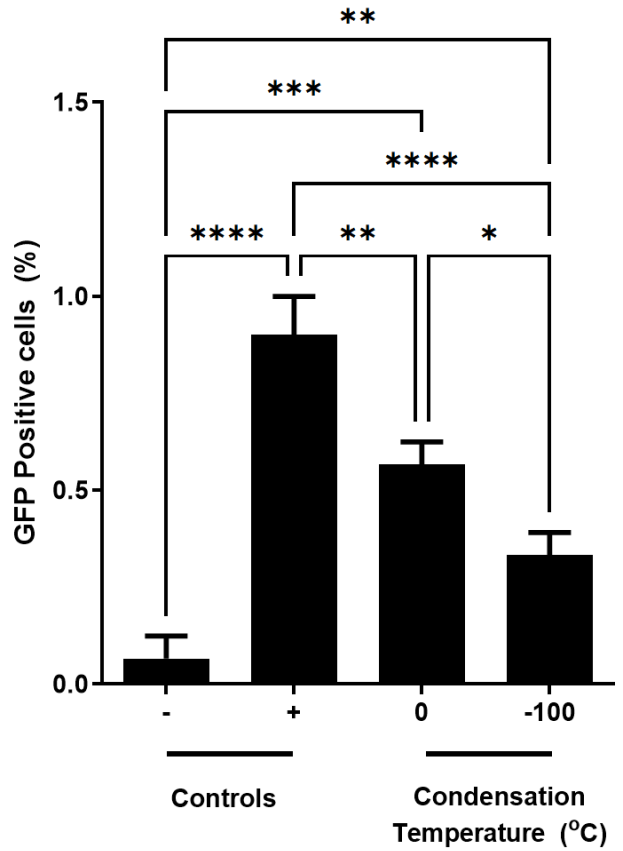

GFP-expressing VSV-pseudotyped lentivirus at MOI 0.01 nebulized using PARI TurboBoy SX and captured using PBM-HALE™. Condensates seeded on 10,000 HEK-293T's and GFP expression measured at 72hrs by fCM

Data by Dr John Henderson

COVID19 Exploratory Pilot Study

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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

Samples:

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

Analysis:

- EBC FA amount
- NP Swab positive by LFT
- Viral load by RT-PCR

COVID19 Exploratory Pilot Study

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CHALLENGE:

- Clinic attendance at week 2+
- Ventilation, health & safety
- HCP Fear
- Pandemic kinetics
- Vaccination
- Clinician compliance

PROGRESS:

- COVID19 ward testing, unventilated
- Admitted COVID19 patients (n=12) all negative in FA
- Acute COVID19 patients to date: n=23, all negative in FA

Is SARS-CoV-2 airborne?

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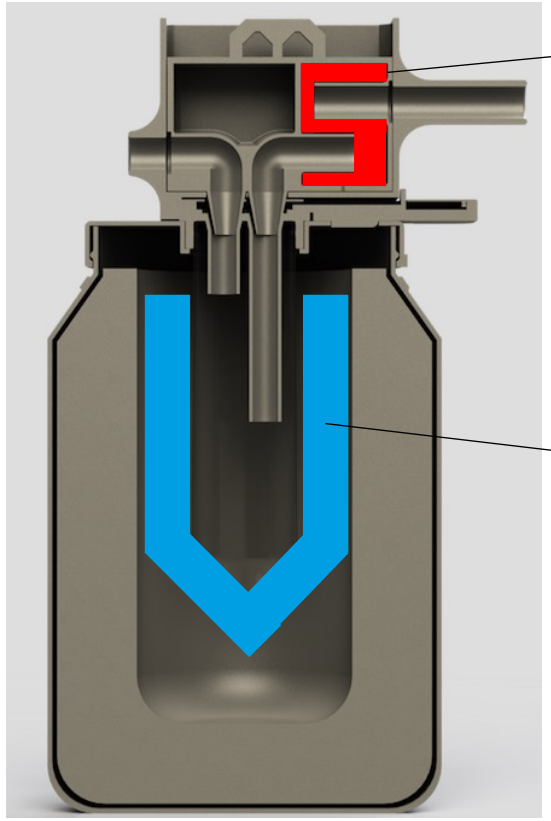

PBM-HALE™

Large droplets: Not Collected

Fine aerosols, Not Detected (n=35)

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

Is SARS-CoV-2 airborne?

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3rd party studies:

- Breath +ve by PCR: 0-93%
- Masks and filters: manual/environmental contamination

Sammadar A. *et al. Open Forum Infect. Dis.* 2021; Williams C. *et al. J. Infect.* 2021; Malik M. *et al. Int. J. Infect. Dis.* 2021

Is SARS-CoV-2 airborne?

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3rd party studies:

- Breath +ve by PCR: 0-93%
- Masks and filters: manual/environmental contamination
- Devices with no salivary separation: positive

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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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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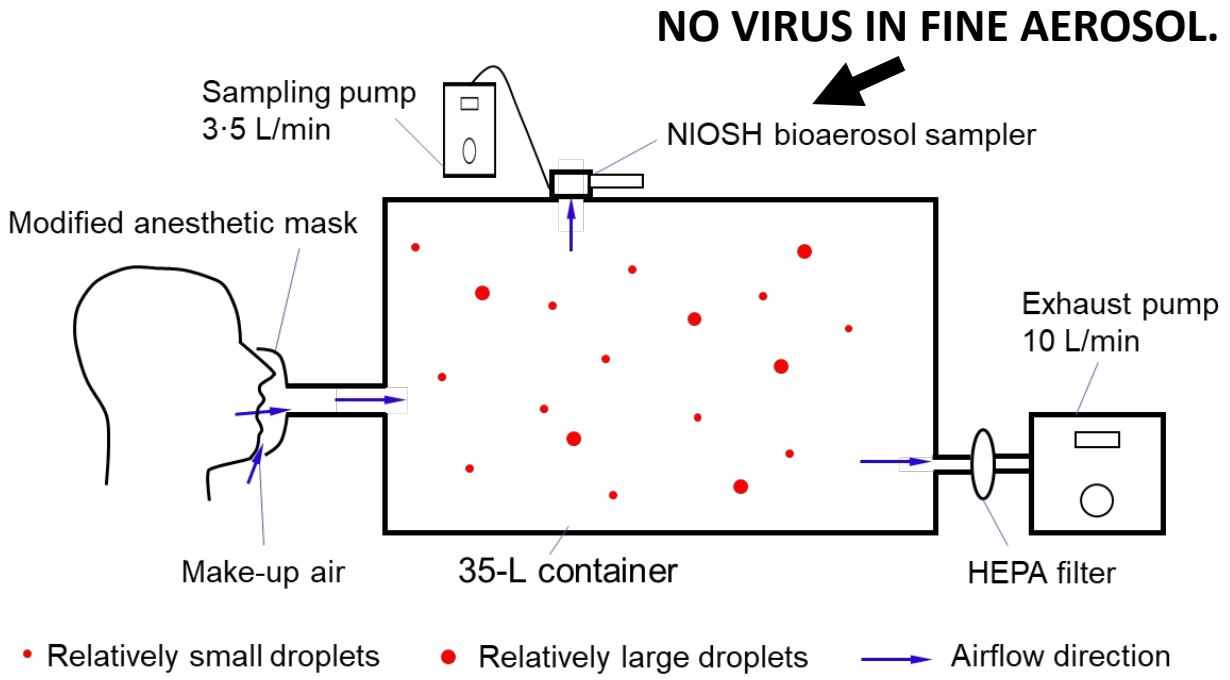

Feng B *et al. J. Hazard. Mater* 2021

Is SARS-CoV-2 airborne?

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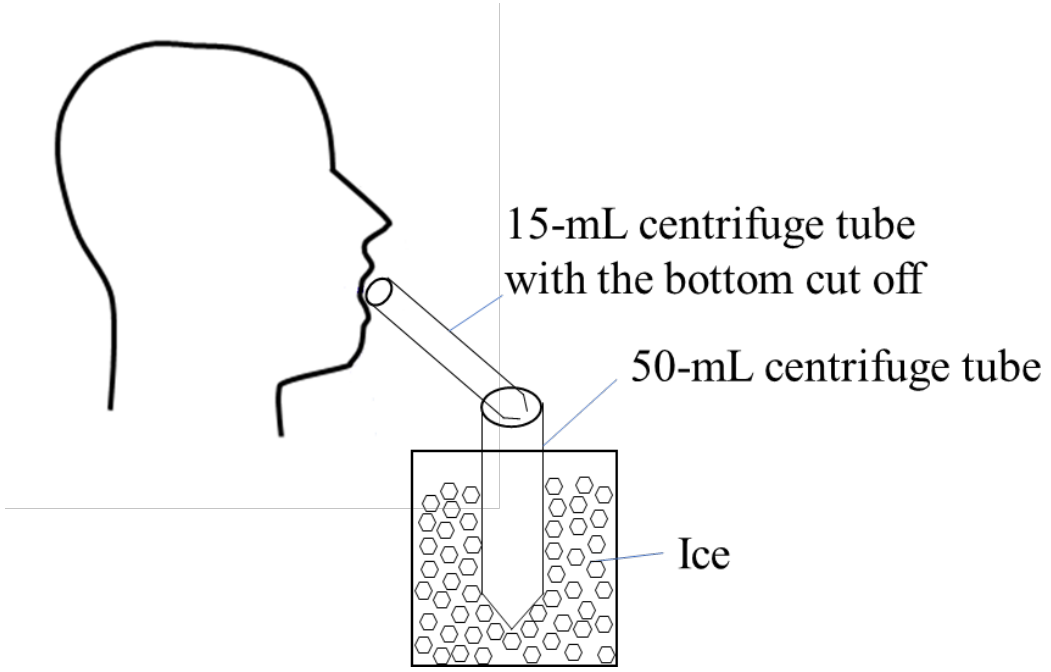

Some positive samples in this device from same study

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

Conclusions

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PBM-HALE™ FA EBC fraction:

- Not contaminated by:
  - Saliva
  - Ambient aerosols
- Cell membrane components
- DNA, RNA, protein
- Drugs of abuse
- Bacteria, fungi
- Distinct microbiome

SARS-CoV-2 in breath:

- Not found in alveolar FA
- Present in saliva
- Present in saliva-contaminated EBC samples
- Respiratory infection pathogen detection will require LD and FA analysis.

Future directions

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Academic

- LD vs FA SARS-CoV-2 study
  - Tidal breath
  - Loud speech
- Methods and reagents for low biomass direct SEQ
- Asthmatic microbiome dysbiosis

Commercial

- PulmoBioMed Ltd.
- Analytical platform partnerships
- Clinical/Pharma researcher use
  - Cancer
  - Idiopathic Pulmonary Fibrosis
  - COPD/asthma
  - Pneumonia

Acknowledgements

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University of Crete
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Dr Diamantis P. Kofteridis
Mr Taxiarchis Nikolouzakis

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