



**Northumbria  
University**  
NEWCASTLE

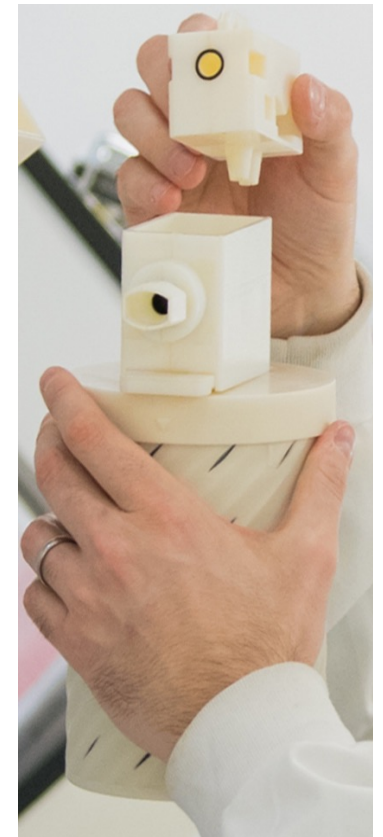
# **Fractionated exhaled breath aqueous particle sampling for diagnostic bioanalytics of COVID19.**

Dr Sterghios A. Moschos FRSC FIBMS FHEA MRSB  
Associate Professor, Cellular and Molecular Sciences  
Founder and Chief Scientific Officer, PulmoBioMed Ltd.  
Twitter: @docmoschos

European Aerosol Conference 2021

# Overview

- Exhaled Breath Condensate for Diagnostics
- The PBM-HALE™ approach
  - Fine Aerosols and Large Droplets at source
  - Microbial capture
  - Bioanalytics data
- Clinical progress update.



PBM-HALE™

# Diagnosing from Exhaled Breath Condensates (EBC)



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

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

RTube™



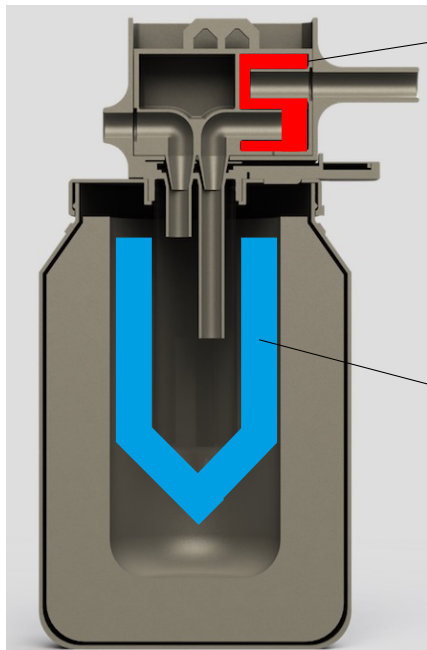
Poor process control

EcoScreen™



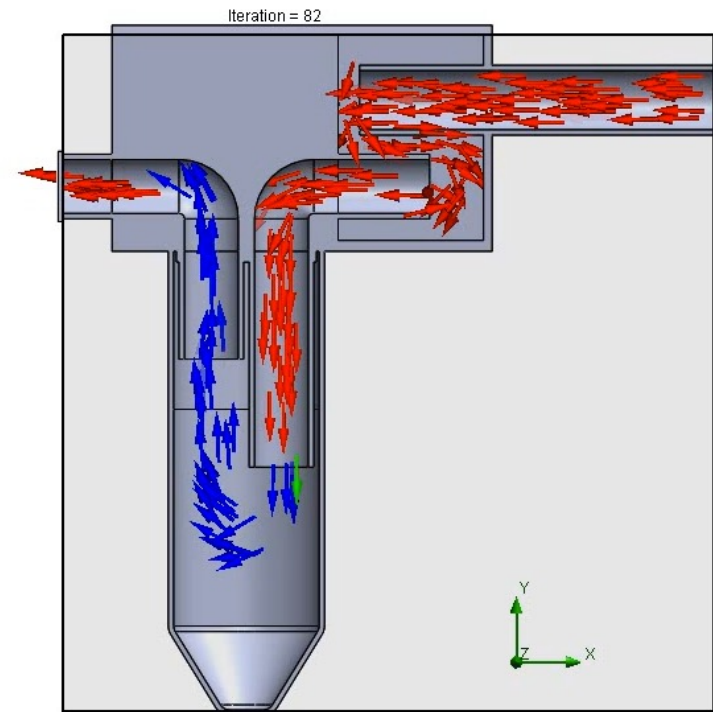
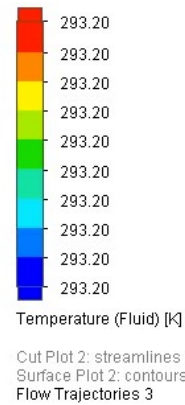
Sample lost in black tube  
17Kg + weight

# PBM-HALE™: the device



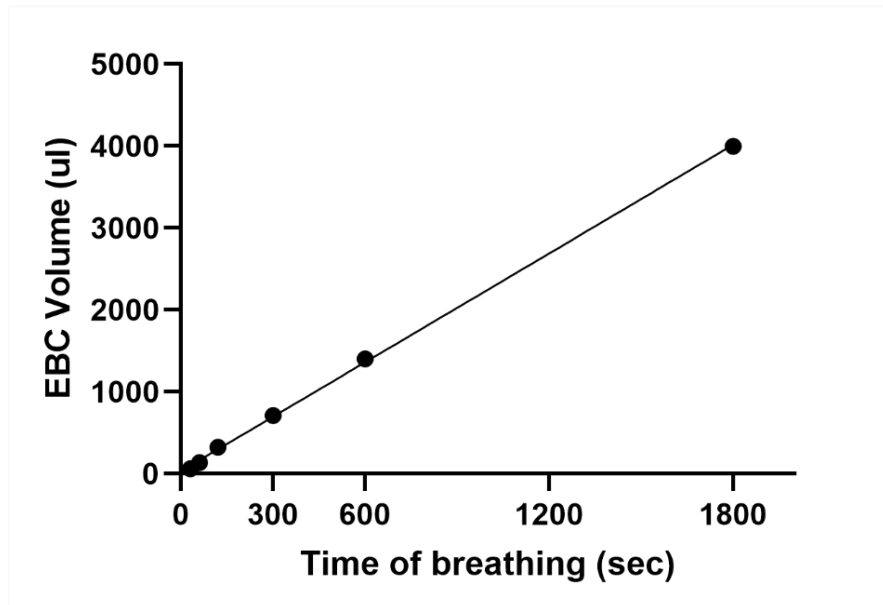
Large droplets  
(LD)

Fine aerosols  
(FA)

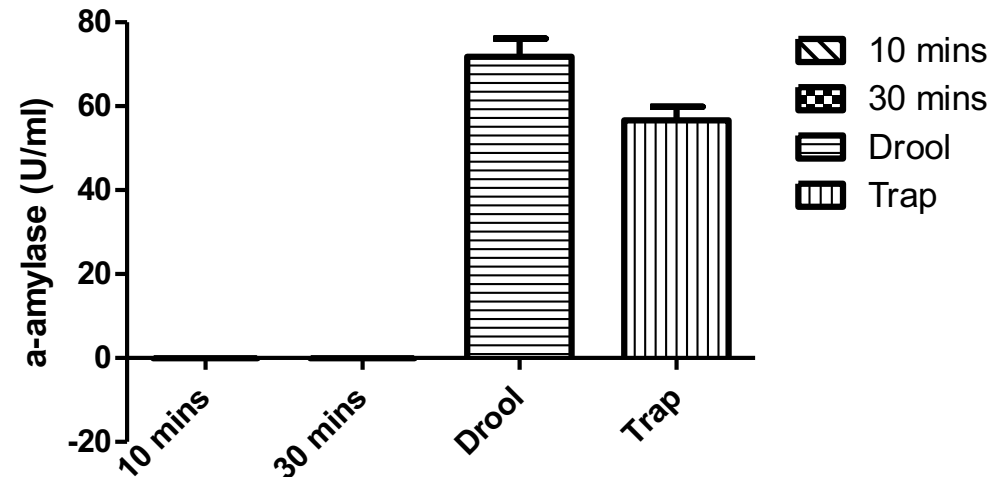


WO2017153755A1: exhaled breath collector – granted; WO2019053423A1: cascade impactor array – granted.

# Performance in FA capture



Production prototype sampling consistency  
0.25 – 30 min  
 $R^2 = 0.9992, n=3$



No salivary amylase in EBC FA  
All volunteer samples to date (n>500)

# Bacteria in EBC FA

Condensation  
Temperature

-ve control

Dry ice (-78.5°C)

Wet ice (0°C)

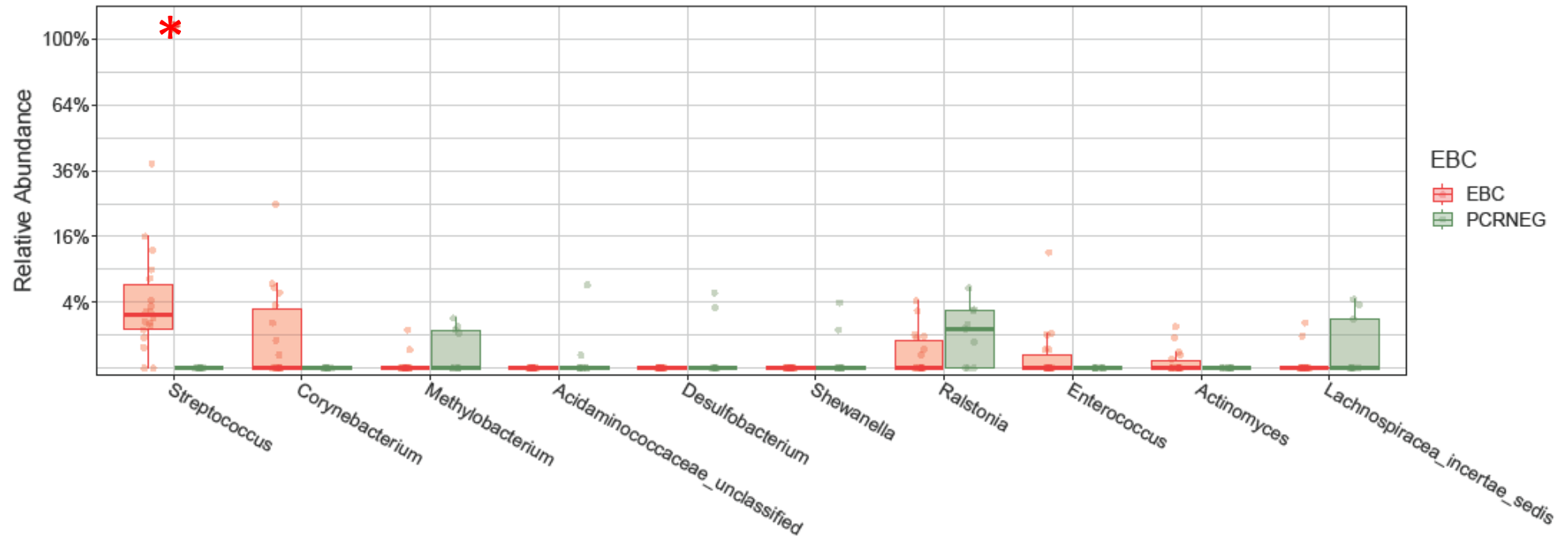
Saliva



2 min sampling period

n = 5, blood agar

# Case study: cause of mild cough



Higher DNA content vs background / kit controls

Detection of *Streptococcus* (FDR  $q = 0.019$ ) w/out extraction in mild cough.



# Metabolomics of EBC FA

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
Palmitoleoylethanolamide	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.

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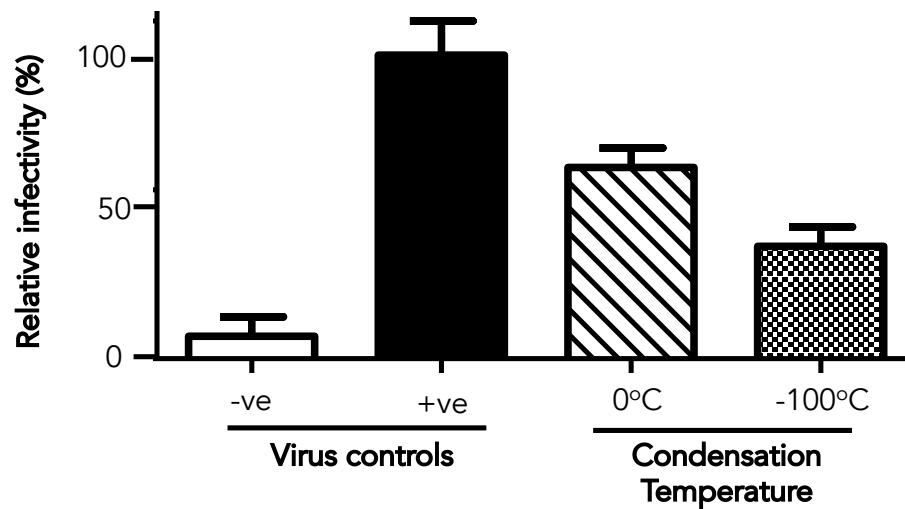
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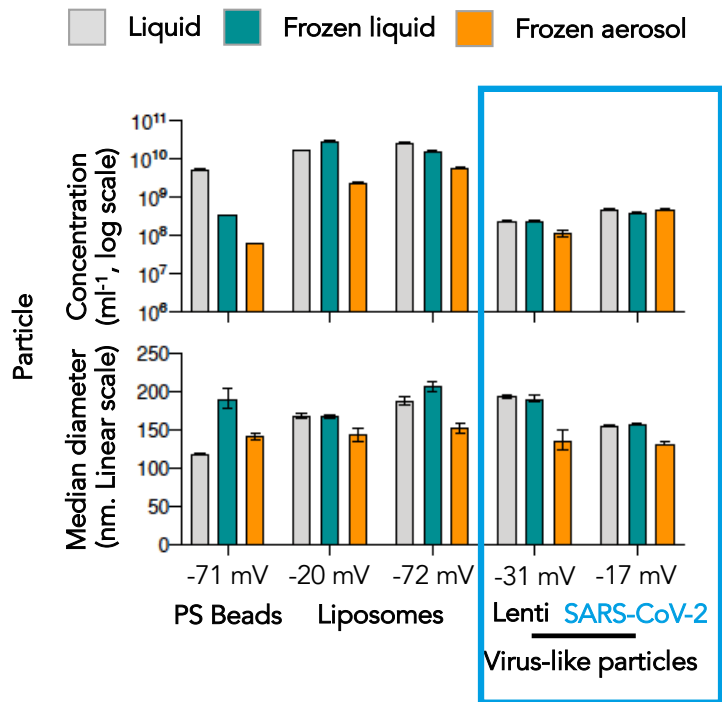
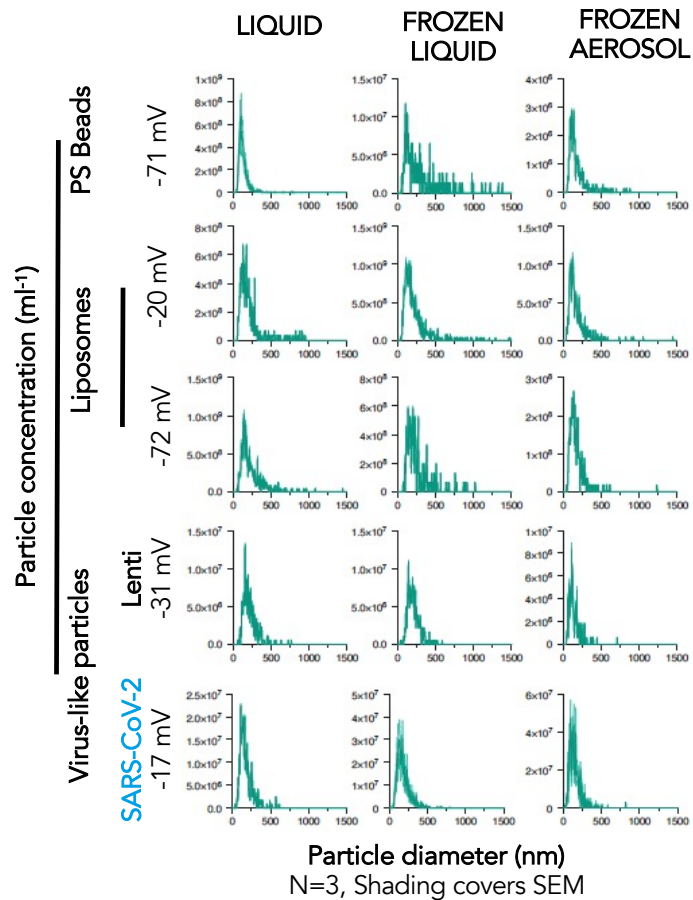
# Infectious virus in mechanical aerosols

25 infectious virions over 15 min sampling.



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

# Aerosolised SARS-CoV-2 Particle Capture



Virtually no loss of VLP size or structure

Aggregation with highly charged particles:

- Drop in concentration
- Rise in particle size
- Beads and liposomes
- Liposome vs VLP stability?

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

# COVID19 Exploratory Pilot Study



## 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
- <30 min
- Aerosol, droplets, NP swab

## Analysis:

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

# COVID19 Exploratory Pilot Study



## NO FALSE POSITIVES:

- COVID19 ward testing, unventilated
- COVID19 patients (n=12).
  - Nasal swab negative.
  - Week 2-3 of symptoms, known nasal -ve period.
- Blinded analysis.

## CHALLENGE:

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

# Conclusions

- EBC FA fraction and LD fraction contain:
  - Bacteria
  - Fungi
  - Cell membrane components
  - DNA, RNA, protein
  - Funky stuff
- PBM-HALE can capture airborne virus
  - Virus in EBC FA remains infectious
  - Significant issues with clinician compliance

# How do I use it?



1. Device use SOP: <https://youtu.be/h6tLt9u-rWU>
2. Lay explanation of use: [https://youtu.be/TkQEj-KN\\_os](https://youtu.be/TkQEj-KN_os)



# Acknowledgements

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