Pulmo BioMed

Your Breath: Your Health™

# Exhaled breath microbiomics and amplicon-based biomonitoring

**Dr Sterghios A. Moschos** FRSC FIBMS FHEA MRSB

Associate Professor, Cellular and Molecular Sciences

Chief Scientific Officer, PulmoBioMed Ltd.

@docmoschos

Northumbria University NEWCASTLE

# Overview

- Exhaled Breath Diagnostics.
  - Background.
  - Hypothesis.
- The PBM-xHALE™ approach.
  - Platform.
  - Microbiomics.
- Clinical progress on SARS-CoV-2.

# Exhaled Breath Condensate (EBC)

- Breath is 95% hydrated.
  - Volatile Organic Compounds.
  - Vapour & aerosols.
  - Biological molecules.

- Biomarker rich:
  - Lung infections.
  - Liver & metabolic disease.
  - Multiple cancers.

# 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

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

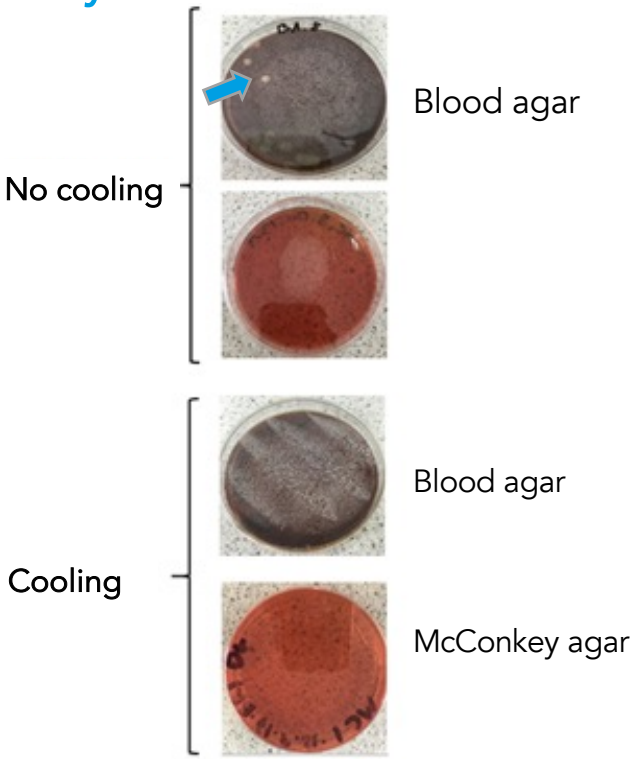
# Exhaled breath condensates (EBC)

- Boyle's law 1662: PV = nRT.
- Dreschel bottle: late 1800's.
- Aerosol physics: 1930's.
- Focus on isolating the sample.
- Eliminate sample loss.
- Separate saliva.

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

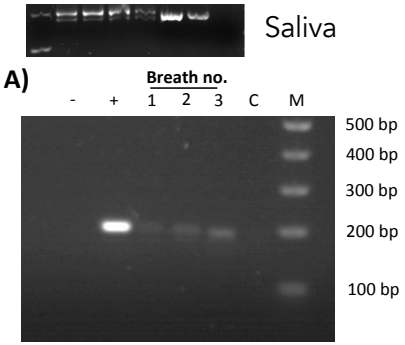
# Preliminary data: bacteria detection

## Bacteria & fungi die by dry ice condensation



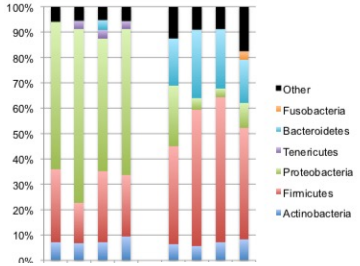

Data by Kavit Shah, Louise Usher

## NAATs work with 1-3 breaths (bacteria)

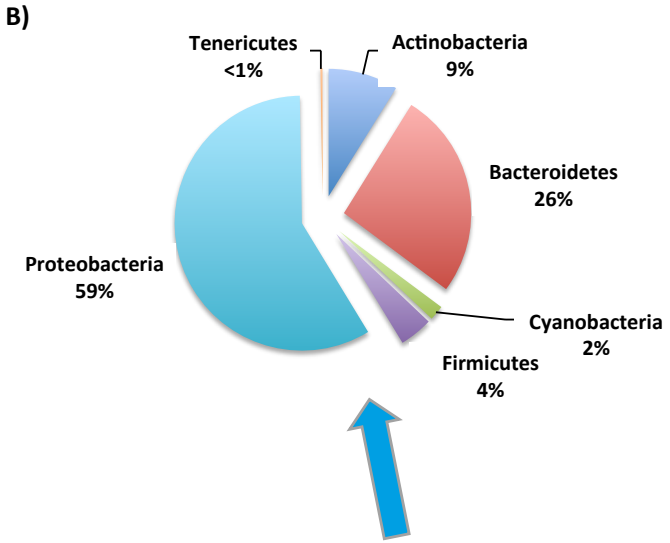



+: positive control (*E. coli* DNA extract)
- : PCR negative control
C : HEPA-filtered air condensate sample
M: 100 bp ladder

## EBC is distinct to saliva



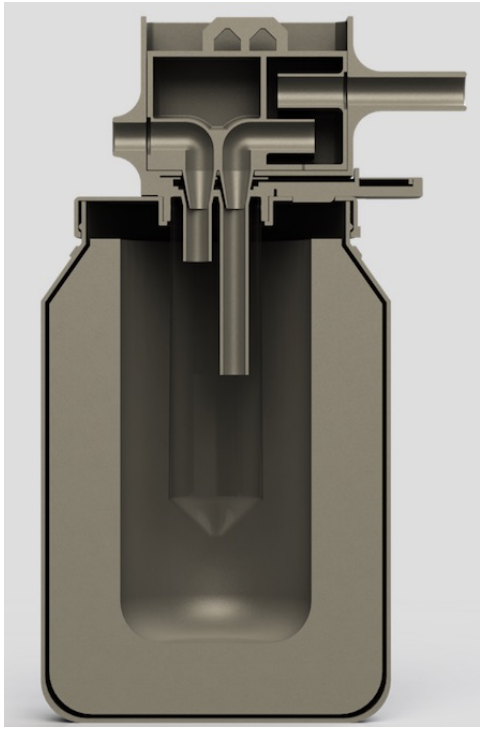

breath saliva




Most similar samples
Come from lung surgery
(60% proteobacteria)

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

# PBM-HALE™: the platform



EBC collector:

- Volatiles and
- Proteins.
- DNA.
- RNA.
- Lipids.
- Medications.

Solves key problems:

- Reproducibility.
- Contamination.
- Sample loss.
- Safety.

**Cold Chain Dependent:**

- Uses dry ice powder ($CO_2$) to collect sample reliably.
- Dry ice replenished every 1 hr from compressed gas cylinder.
- Sample needs on the spot test or frozen transfer to lab.

**WO2017153755A1:** exhaled breath collector – national phase; WO2019053423A1: cascade impactor array – PCT.

# PBM-HALE™: the platform

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EBC collector:

- Volatiles and
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- DNA.
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- Medications.

Solves key problems:

- Reproducibility.
- Contamination.
- Sample loss.
- Safety.

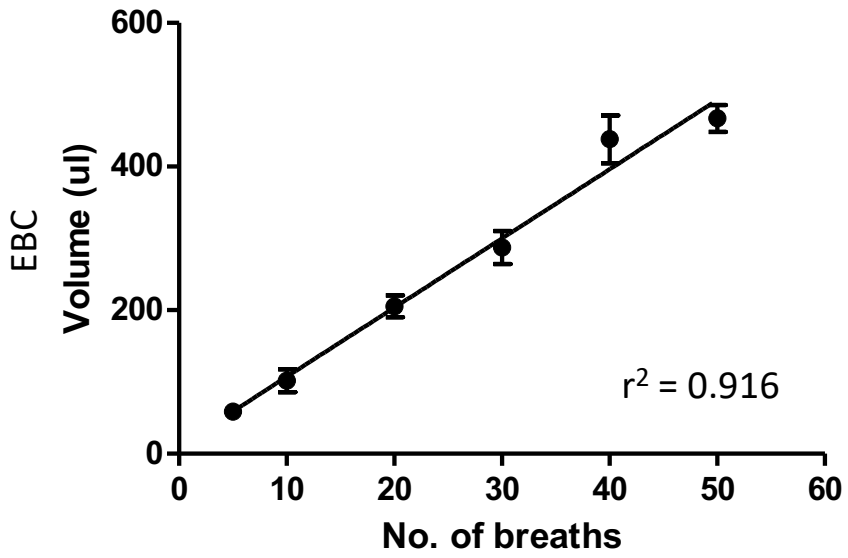
## Path to removing the cold chain:

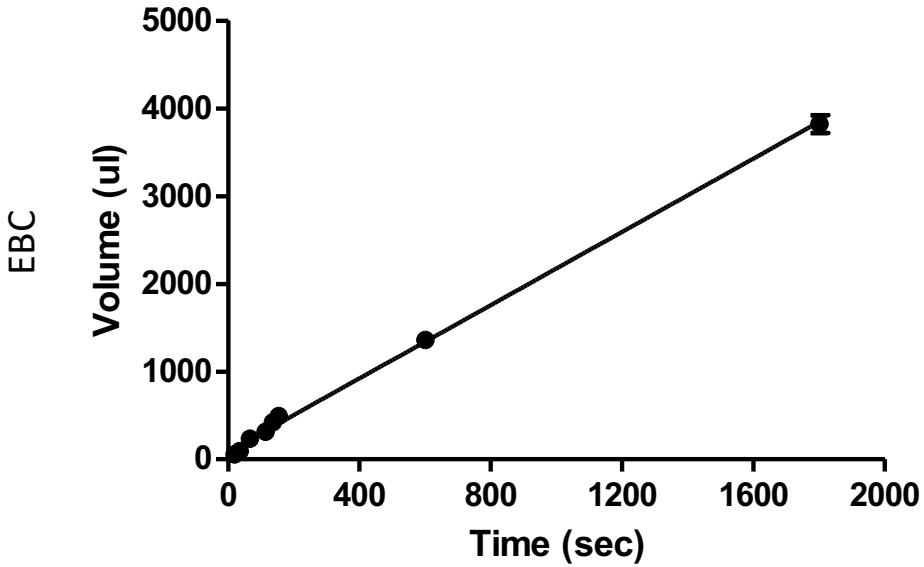
- Proprietary coating to remove need for dry ice.
- Stabilisation material to remove freezer storage.

Experiments under way

WO2017153755A1: exhaled breath collector – national phase; WO2019053423A1: cascade impactor array – PCT.

# Highly consistent sampling



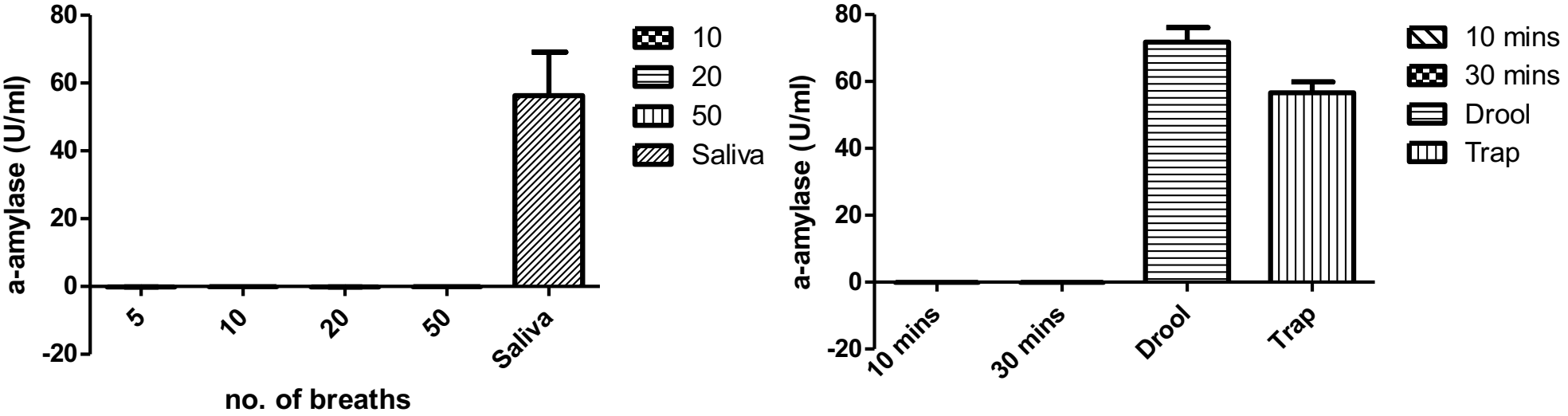




Sampling linear from 25 sec (screening) to 30 min (discovery)

$R^2$ range: 0.88 to 0.95, n = 5.

Data by John Henderson

# No salivary contamination




Saliva enzyme levels at least 5000x above assay limit of detection

n = 5.

Data by John Henderson

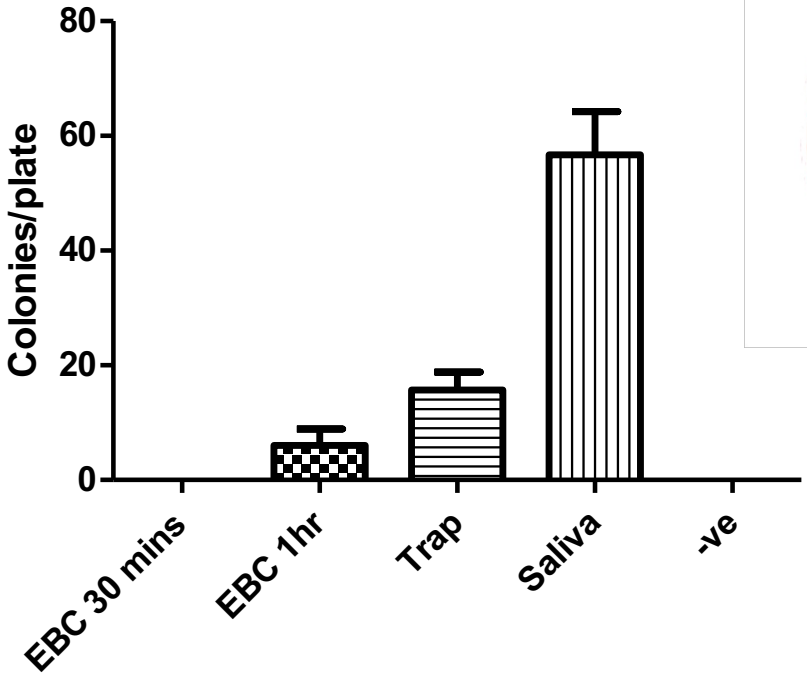
# No bacterial viability


2 min sampling period

n = 5, blood agar

Data by John Henderson

# No bacterial viability

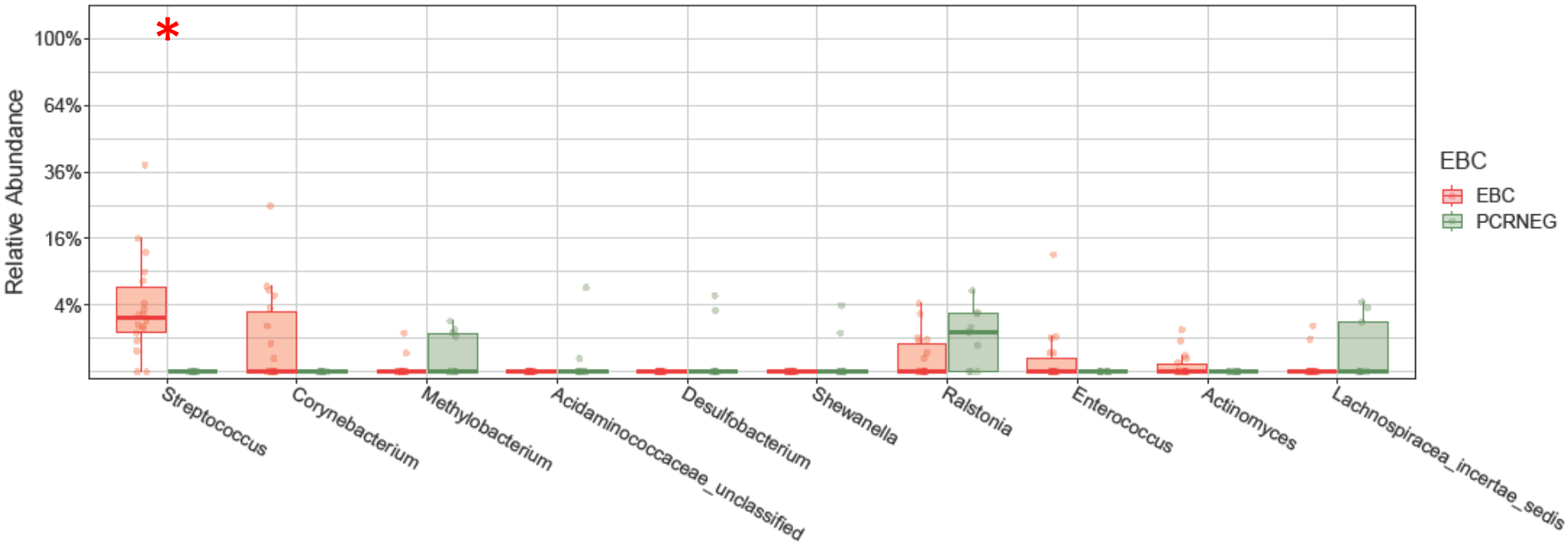




Cooling efficiency lost after ~40 min continuous sampling.
n = 3.

Data by John Henderson

# 16S Microbiomics




Higher DNA content vs background / kit controls

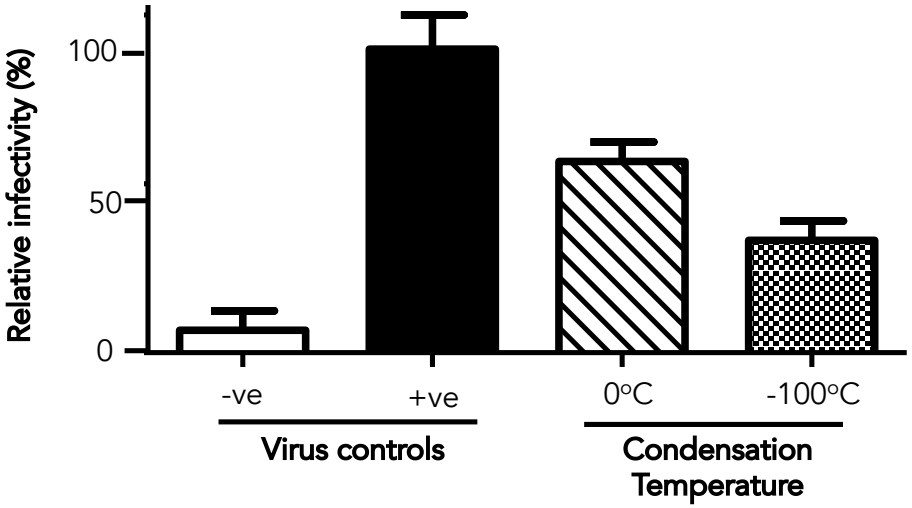
Detection of *Streptococcus* (FDR q = 0.019) w/out extraction.

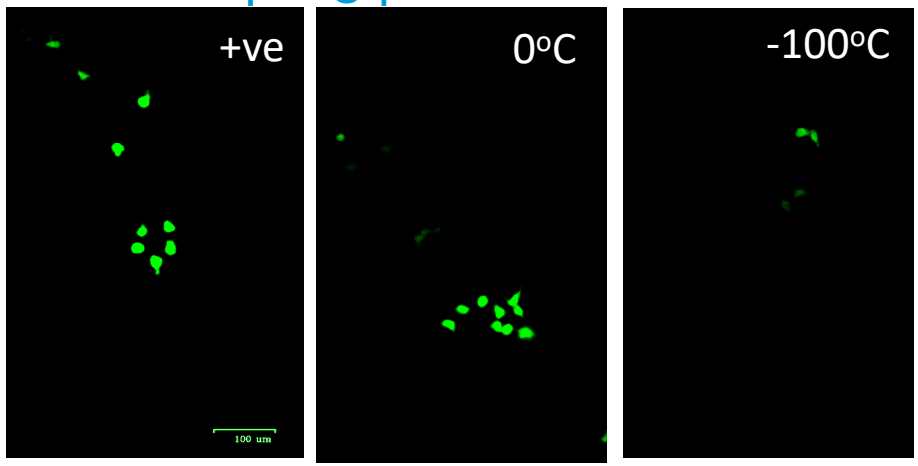
Data by John Henderson, Andrew Nelson

Data generated at the Northumbria University NUOmics Core Service

# Aerosolised viruses

Efficient capture of aerosolized virus; dry ice halves infection risk.
25 infectious virions over 15 min sampling period.

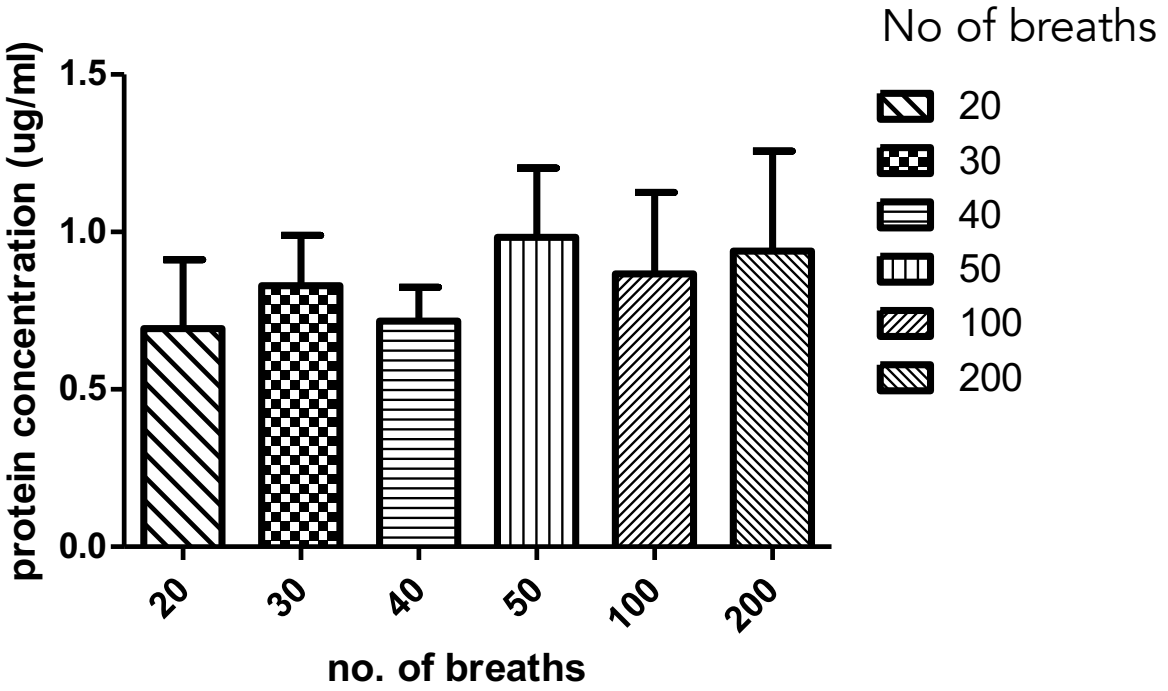



GFP-expressing VSV-pseudotyped lentivirus at MOI 0.01 nebulized using PARI TurboBoy SX and captured using PBM-HALE™. Condensates seeded on 10,000 HEK-293T's and GFP expression measured at 72hrs by FACS, visualized by fluorescent microscopy. Bar = 100 um.

Data by John Henderson

# Consistent [protein]

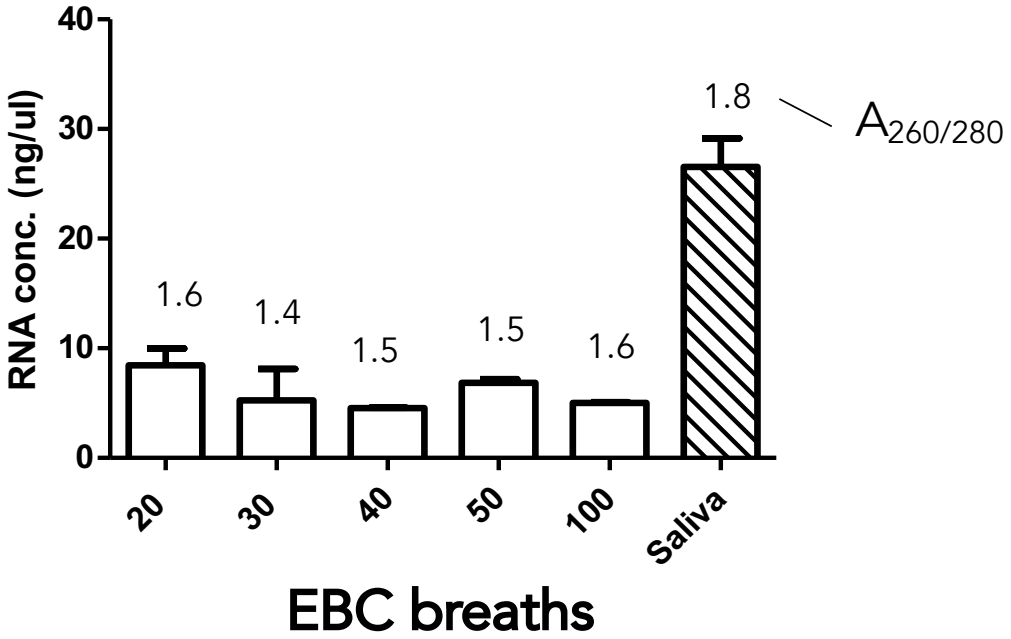



Requires 5x concentration by lyophilization

n=5

Data by John Henderson

# Consistent [RNA]



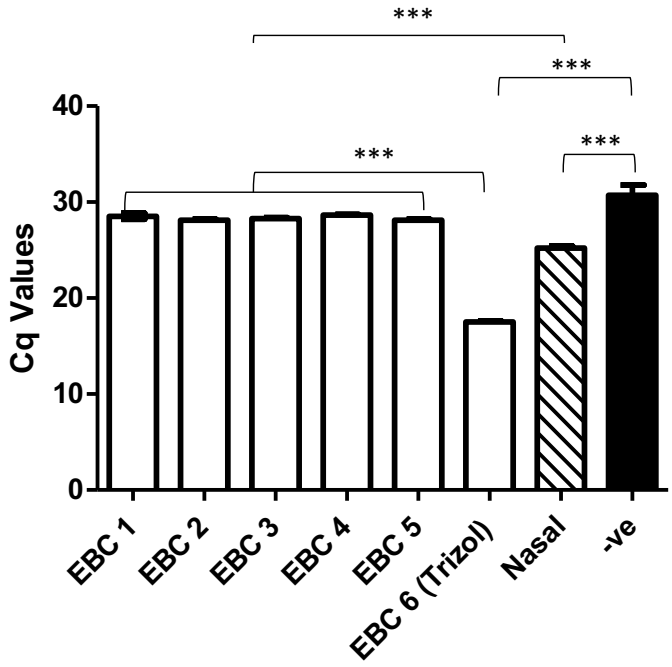

Increased EBC sampling does not increase [RNA].

EBC volume extracted by Trizol matched with volume obtained after 20 breaths.

n=6

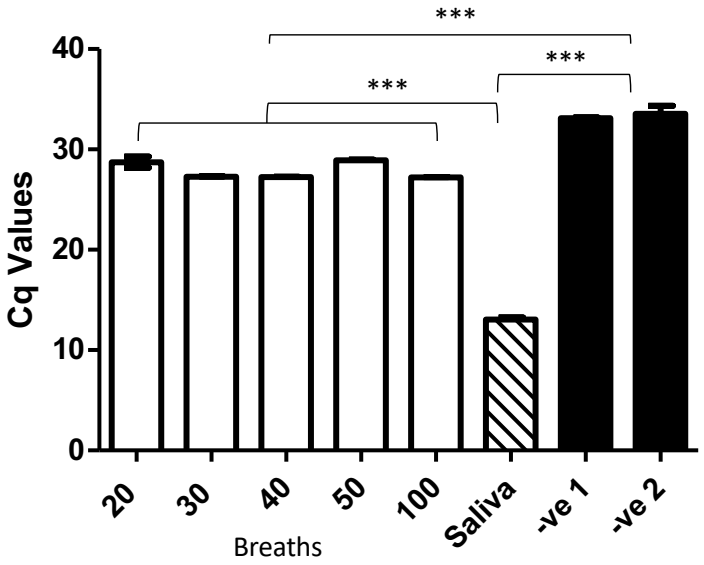
Data by John Henderson

# 18S RNA



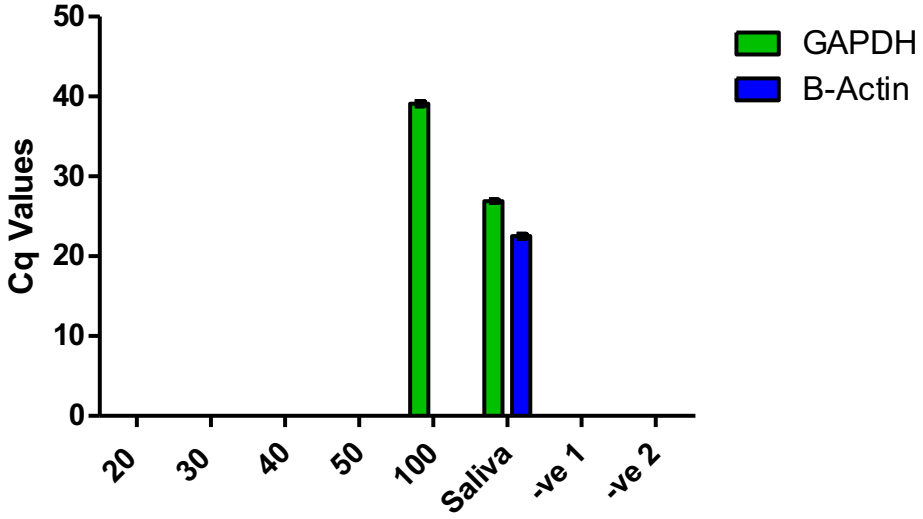

2 step SYBR Gold RT-qPCR (triplicate)
EBC1-5: RNeasy kit 20 breaths
EBC6: Trizol 30 min sample
Nasal = swab.

Data by John Henderson




2 step SYBR Gold RT-qPCR (triplicate)
-ve 1: No RT control
-ve 2: no cDNA
EBC volume normalized to 20 breaths

# GAPDH & β-actin RNA

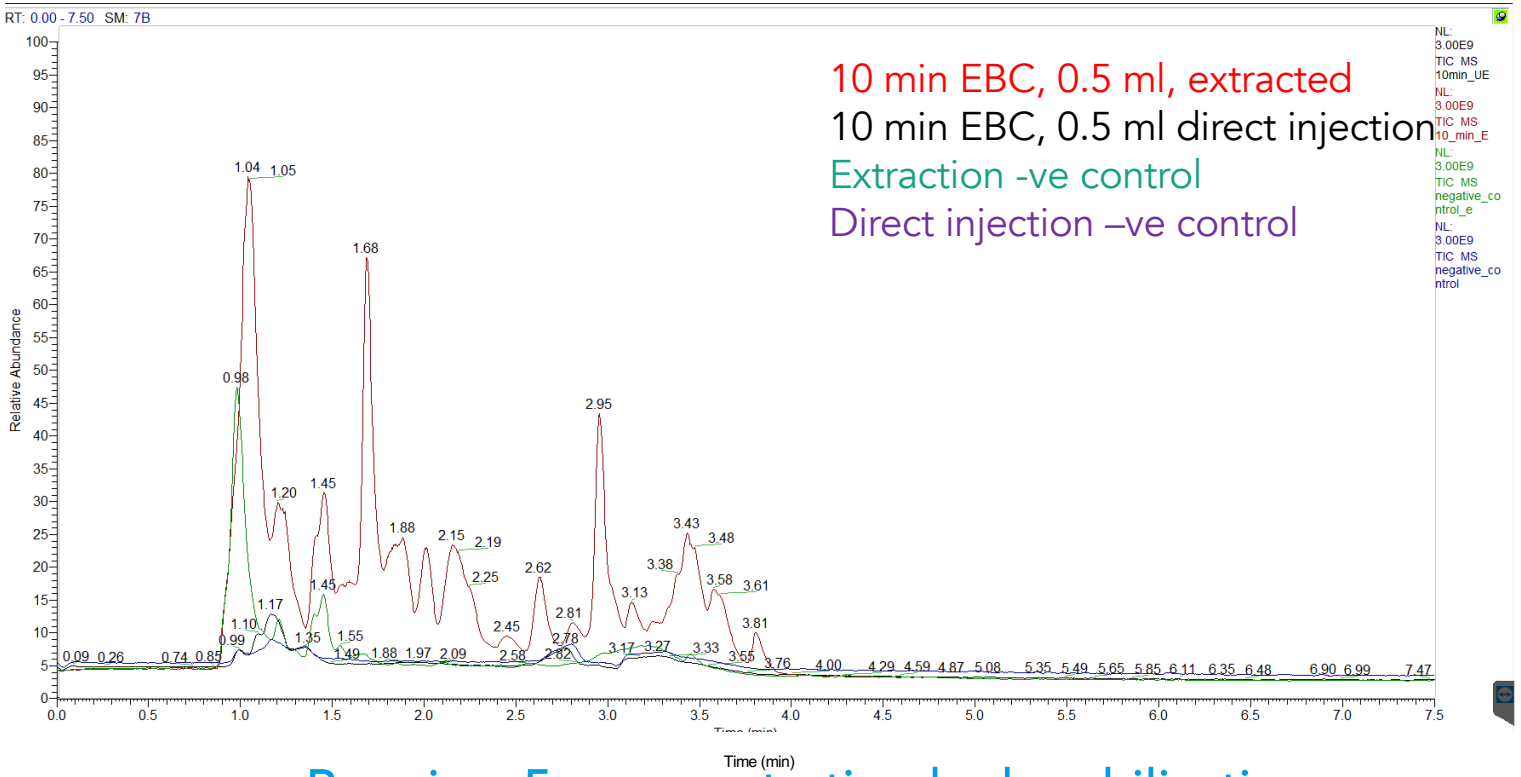



**2 step SYBR Gold RT-qPCR (triplicate)**
-ve 1: No RT control
-ve 2: no cDNA
EBC volume normalized to 20 breaths

Either low host [RNA] or 18S all fungal.
n=5

Data by John Henderson

# Metabolomics




Requires 5x concentration by lyophilization

n=5

Data by John Henderson, William Cheung

Data generated at the Northumbria University Metabolomics Core Service

# Metabolomics

| 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 |

Data generated at the Northumbria University Metabolomics Core Service

## 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.*

# COVID-19 Pilot data.

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- <30 min EBC (3-4 ml)
- n=60 target.
- n=35 interim.
- 6-10 centres.
- In COVID19 red zone/ward.
- Use local Dx RT-PCR assay.
- Blinded sample Dx analysis.

- Target population:
  - Any age group/sex.
  - Week 1 of symptoms.
  - Nasal positive (<24 hrs).
  - Focus on symptomatic close contacts of COVID-19 cases.

**NO FALSE POSITIVES OR FALSE NEGATIVES TO DATE (n=6 controls).**
**30-60 sec appears adequate.**

Clinical data as of 03/11/20

# SARS-CoV-2 in exhaled aerosols?

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- **Fast:** <1 min sample.
- **Simple**: just breathe.
- **>2x safer**: process halves infectivity.
- **More reliably**: Tuneable sample vs nasal swabs.

- **For mass screening**: by mass production of plastic.
- **Where patients are:** using *any* point of need testing system.
- **With current gold standard tests** (QuRapID®[1], ID NOW®).

1. Shah K. *et al. Chem. Sci.* 2017

# Next steps

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## Research

- Eliminate cold chain.
- Detect virus by LFT / aptamers.
- On-board analytical capability.

## Development

- Spinout trading 19/10/20.
- MHRA LNO / FDA IDE; EUA.
- Mass production, distribution.
- Adaptations for environmental sampling.

**ALL DEVICES PRODUCED THROUGH CHARITABLE/GOVERNMENT FUNDING WILL BE MADE FREELY AVAILABLE FOR COVID19 PANDEMIC**

# Acknowledgements


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Miss Zoe Hewitson

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Dr. Jan Münch

**University of Oxford**
Prof. Aris Katzourakis

**PulmoBioMed Ltd.**
Dr. Pete Hotten (CEO)
Dr. Huw A. Edwards (Chairman)
Mr. Jonathan Brooks (COO).
Mr Saqib Ali

Consultancy disclosures:

# 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