UPDATED: 5th May 2021

Pulmo BioMed

Your Breath: Your Health™

Coronavirus 19

Understanding Breath Aerosol Transmission Risk

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@docmoschos

Northumbria University NEWCASTLE

Overview

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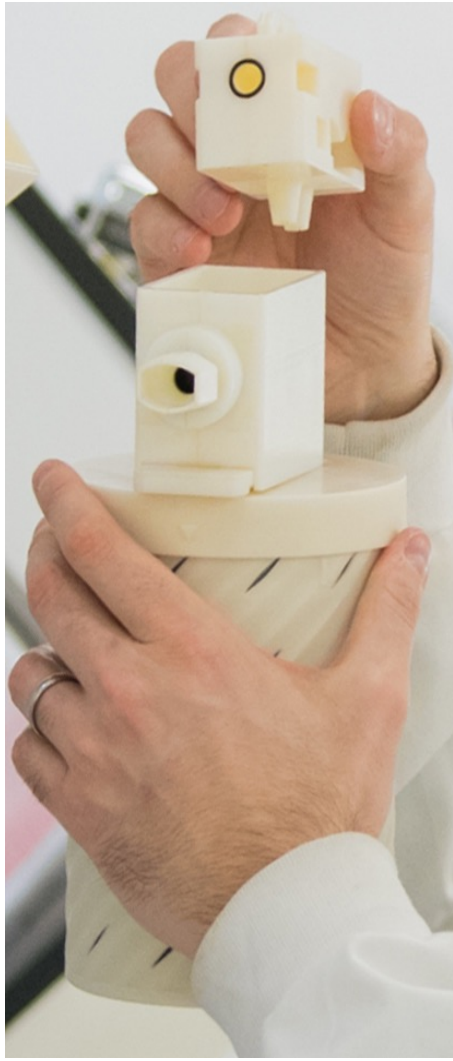
- Breath for Coronavirus screening.
  - Scientific evidence.
  - Key unknowns.
- Exhaled Breath Diagnostics.
  - Opportunities.
  - Challenge.
  - Competition.
- The PBM-HALE™ approach.
  - Platform IP.
  - Supporting key data.
- The proposition.



PBM-HALE™ (MVP)

Coronavirus 19 (COVID-19) in breath: Confidence in rationale

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Biology

- SARS-CoV-2 binds ACE2 receptor.[1]
- ACE2 protein levels highest in lower lung.[2]
- Aerosols (<5 μm) best to reach lower lung (drug delivery science).[3]

Pathology

- Disease of the lower lung: respirator need.
- Proposed transmission routes: fomites, droplets (cough, >5 μm), but:
  - Models & data[4] show transmission without symptoms (no cough!).
  - Aerosol science used in epidemiology out of date.[5]

1. Hoffman M. *et al. Cell* 2020; 2; Hamming *et al. J. Pathol.* 2004; 3. Moschos SA *et al. Pharm. Thera.* 2017; 4: Tindale LC *et al. MedRxiv 2020;* 5. Bourouiba L. *JAMA* 2020.

Coronavirus 19 (COVID-19) in breath: Confidence in rationale

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Clinical evidence

- Transmission occurs up to 1 week before symptoms (peak @ -2.9 days).[1]
- Virus genome levels max in lower lung samples > nose > throat.[2-5]
  - <42% false negative oral swabs.
  - 10-25% false negative nasal swabs.
  - Viable virus levels low in nasal swabs.
  - Nasal detection ~70% days 0-5 from symptoms[6]

Experimental evidence:

- COVID-19 ward aerosol gel traps –ve, but ceiling air vents +ve:[7] Droplets pulled by gravity, aerosols pulled by air flow.[8]
- Aerosolised virus infectious for 16hrs after mechanical generation.[9, 10]
- Other coronaviruses can naturally aerosolize (n=3000).[11]

1. Tindale LC *et al. MedRxiv* 2020; 2: Winnichakoon P. *JClinMicro* 2020; 3: Wu *et al Clin Inf Dis* 2020; 4: Yang Y. *et al. MedRxiv* 2020; 5: Ai T. *et al. Radiology* 2020; 6: Zhao J. *et al.* Lancet 2020; 7: Ong SWX *et al.* 2020; 8: Bourouiba L. *JAMA* 2020. 9: Holbrook MG *et al. NEJM* 2020; 10. Fears AC *et al.* MedRxiv 2020; 11. Leung N.H. *Nature Med.* 2020.

Coronavirus 19 (COVID-19) in breath: Confidence in rationale

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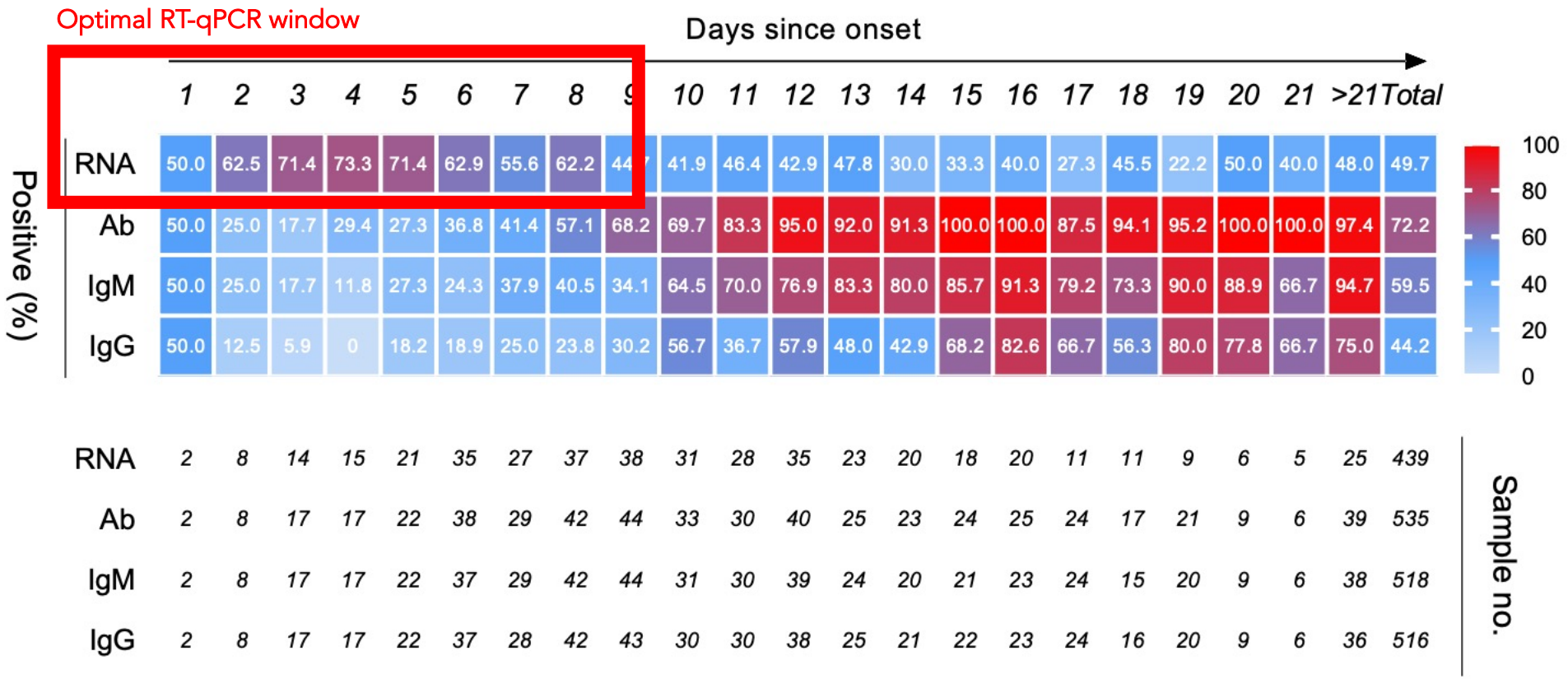

Detection of SARS-CoV-2 in nasal swabs (RNA) or blood (IgX) from symptom onset

Zhao J. *et al.* Lancet 2020.

Coronavirus 19 (COVID-19) in breath: Confidence in rationale

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Our hypothesis

- Disease is a function of amount of virus reaching the lower lung.
- Achieved mainly by breath aerosols (or poor immune system).
- Explains close contact transmission chains.

We need to test breath aerosols for:

- The amount of virus present (genomes).

- Infectivity (viruses).

Diagnosing from Exhaled Breath Condensates (EBC)

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Breath is 95% hydrated:

- Volatile compounds (smells, eg garlic, alcohol).
- 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

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 platform

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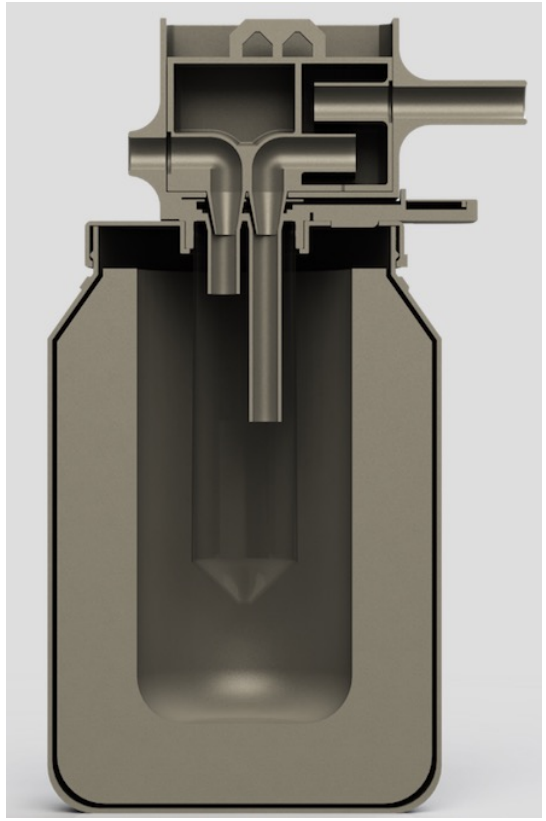

PBM-HALE™
(TLR8 RUO/POC/PON use)

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 – granted; WO2019053423A1: cascade impactor array – granted

PBM-HALE™: the platform

PBM-iHALE™
(TLR 5 PON use)

EBC collector:

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

Preliminary data: pathogen DNA

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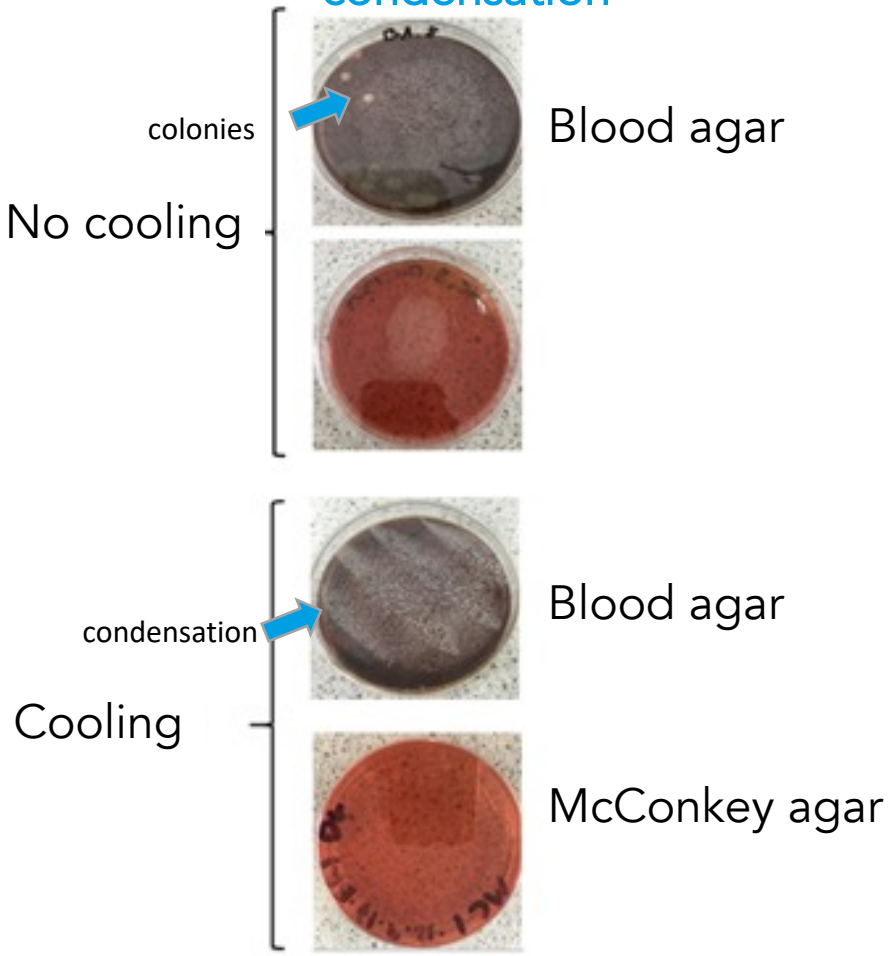
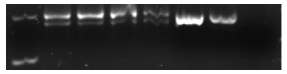
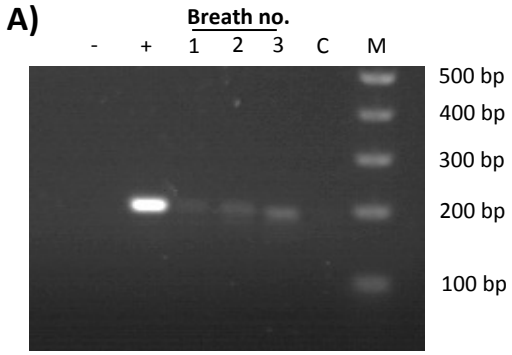
Bacteria & fungi die by dry ice condensation



NB: no colonies on plates, white marks are condensation

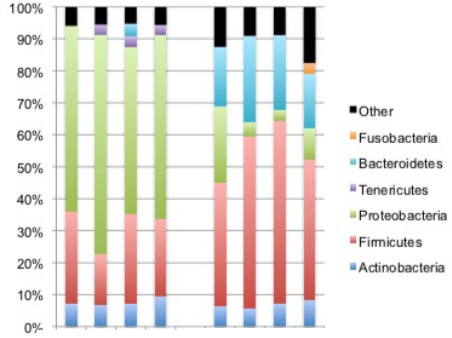
COVID-19-like test works with 1-3 breaths for bacteria


Saliva

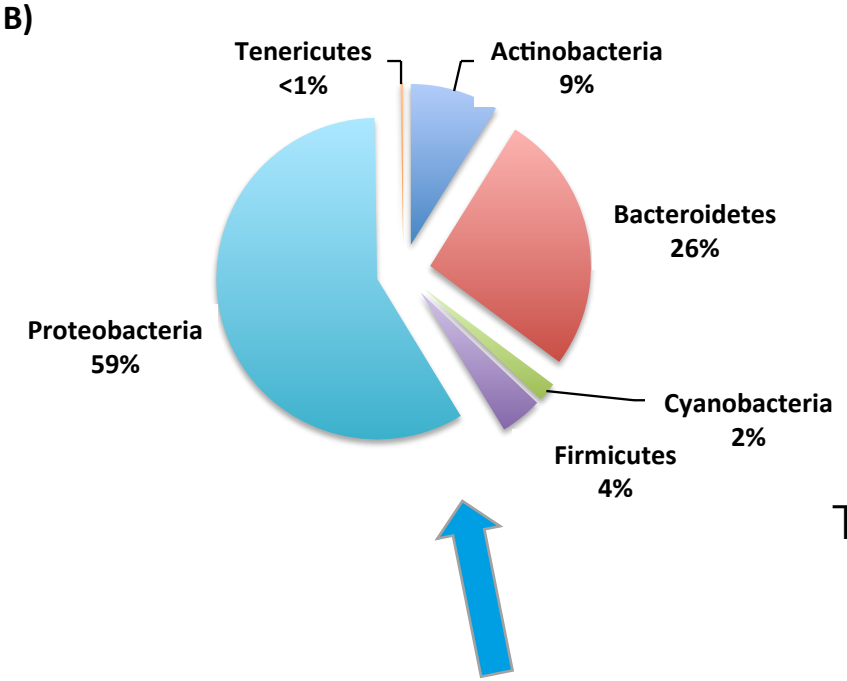


+: positive control (*E. coli* DNA extract)
- : PCR negative control
C : HEPA-filtered air condensate sample
M: 100 bp ladder

EBC is distinct to saliva



breath saliva




Tool device

Closest sample like this comes from lung surgery samples only (60% proteobacteria)
(Sze MA *et al. Am. J. Resp. Crit. Care Med.* 2012)

Amie Wilkinson, Kavit Shah, Louise Usher, Zoe Hewitson
U. Westminster, U. Northumbria

Prototype: highly consistent sampling

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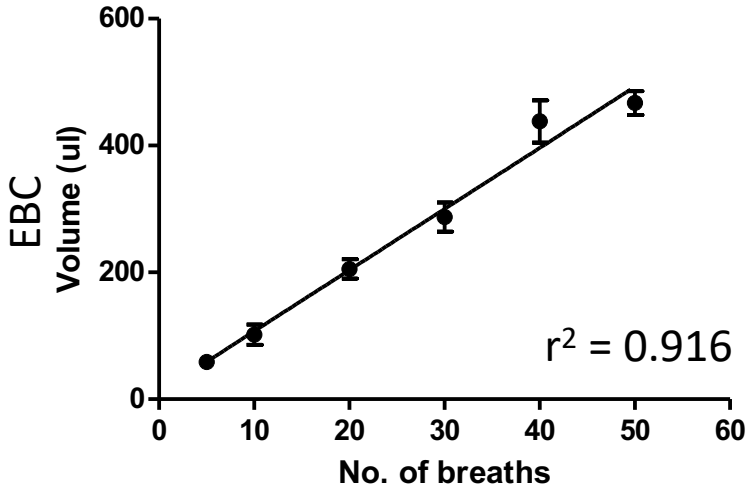

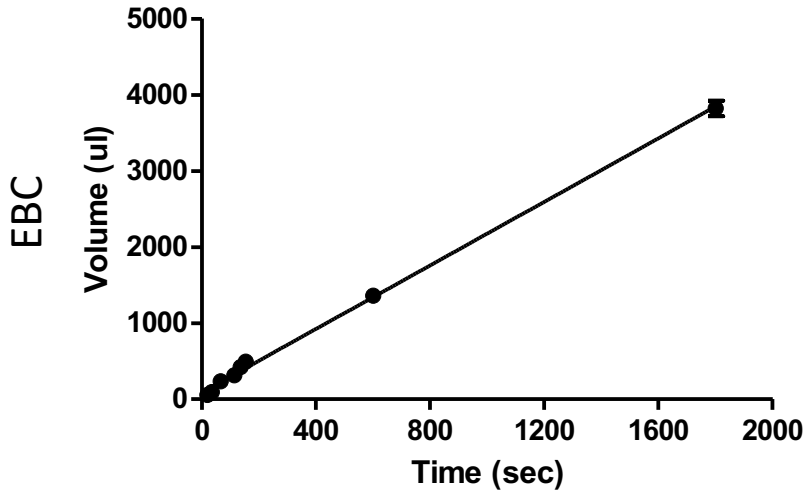

Whether 5 breaths (25 sec; e.g. screening) or 30 min of sampling (e.g. discovery)

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

John Henderson
U. Northumbria

Prototype: no salivary contamination

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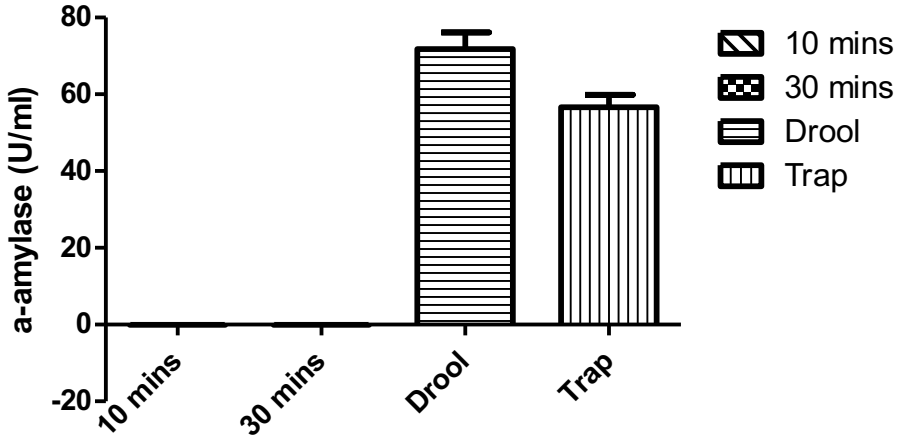

Saliva enzyme levels below limit of detection in EBC:

At least 5000x less in EBC than in saliva (drool)) or device saliva trap levels even after 30 min sampling.

n = 5.

John Henderson, Zoe Hewitson
U. Northumbria

Prototype: No microbial growth due to dry ice condensation (blood agar).

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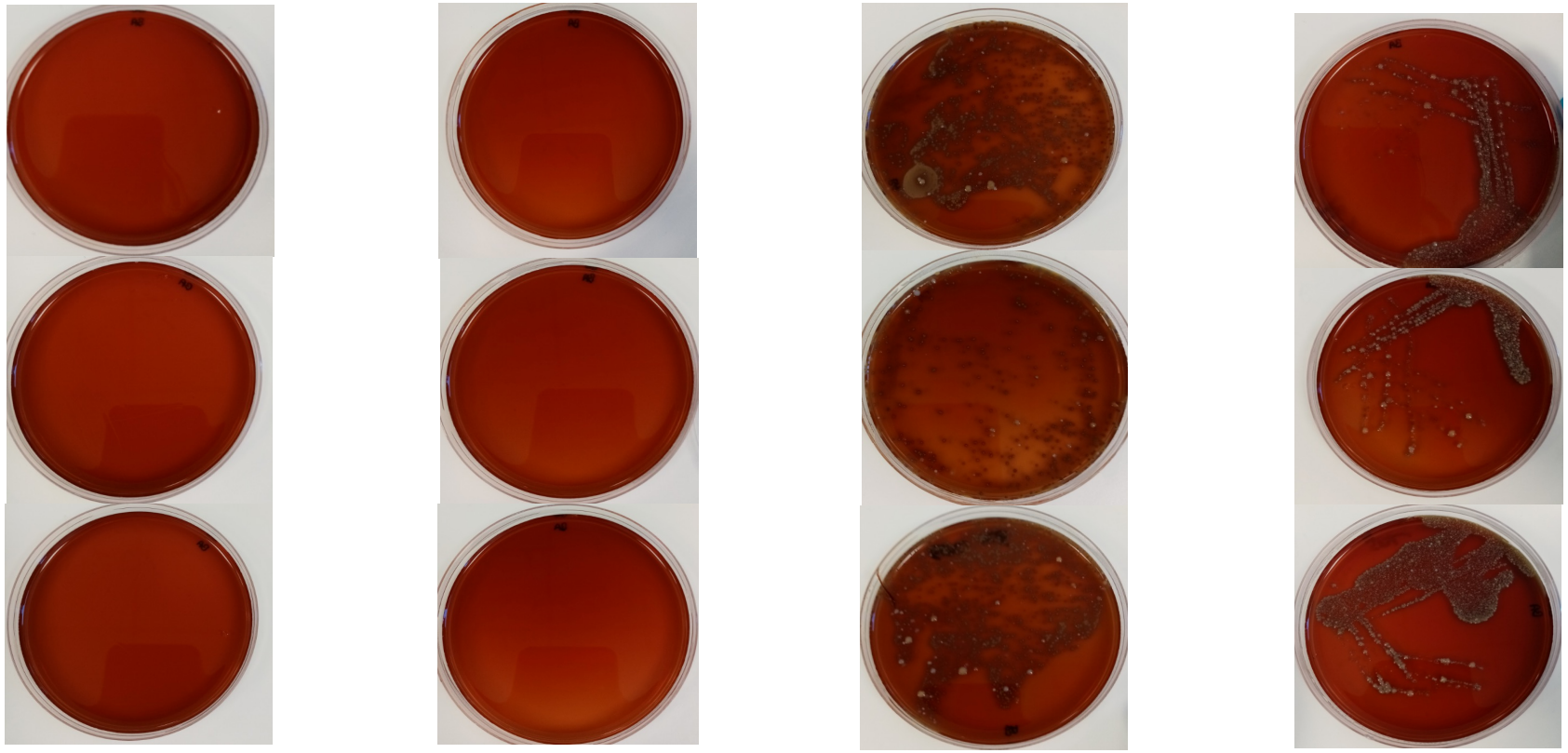
Condensation

-ve control | Dry ice (-100°C) | Wet ice (0°C) | Saliva



2 min sampling period

(2x target sampling period for COVID-19 screening use).

n = 5

John Henderson, Zoe Hewitson
U. Northumbria

Prototype: microbial growth only after loss of dry ice cooling efficacy

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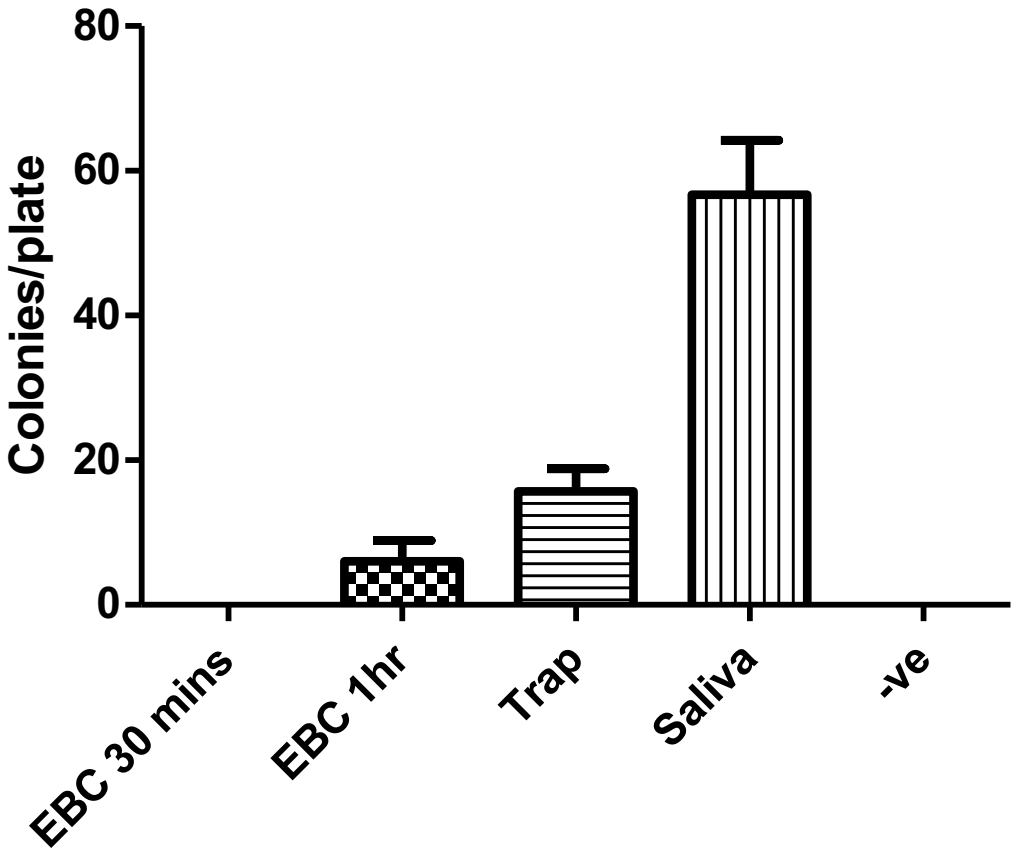
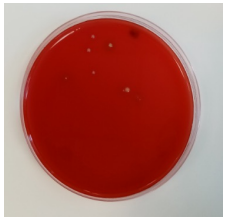
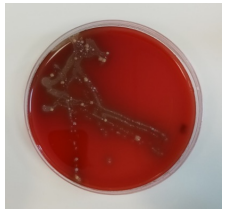


EBC 1hr



Saliva Trap

Saliva control



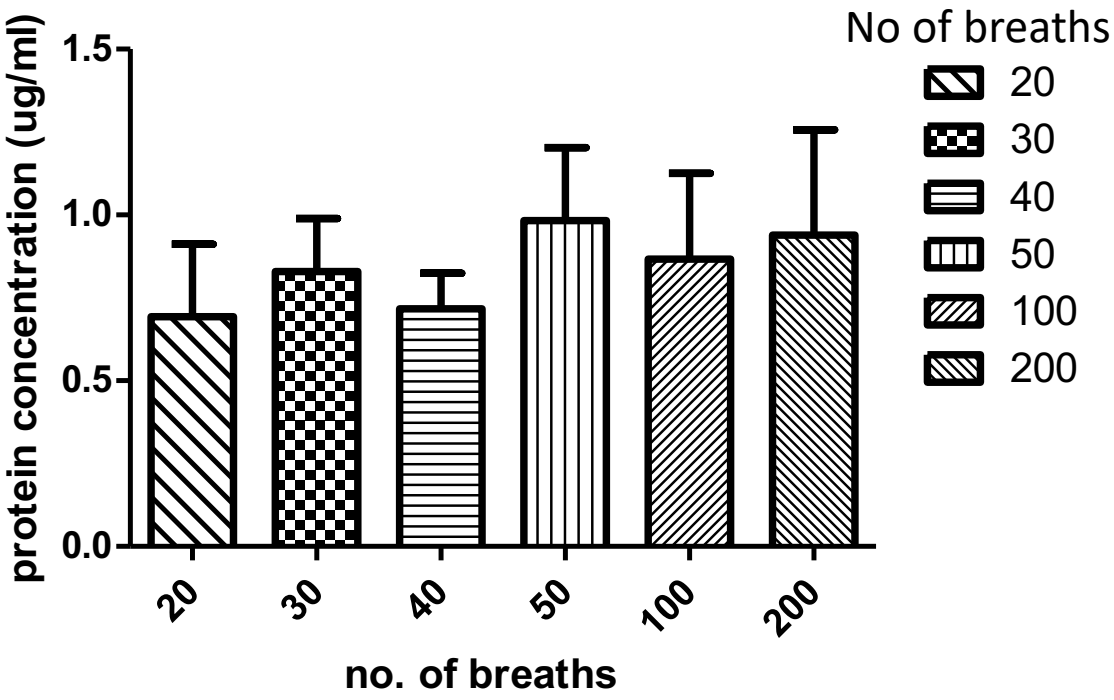
Colonies from dry ice-captured EBC cultured on blood agar.

Lateral contact of sampling tube to dry ice lost ~40 min after continuous sampling.

n = 3.

John Henderson
U. Northumbria

Prototype: consistent [protein] in EBC



Samples lyophilized and re-constituted in 1/5th of original volume:
No statistically significant difference in concentration over time by micro BCA (data close to LLOD).
No concentration increase anticipated.
n=5

John Henderson
U. Northumbria

Prototype: consistent [RNA] in EBC

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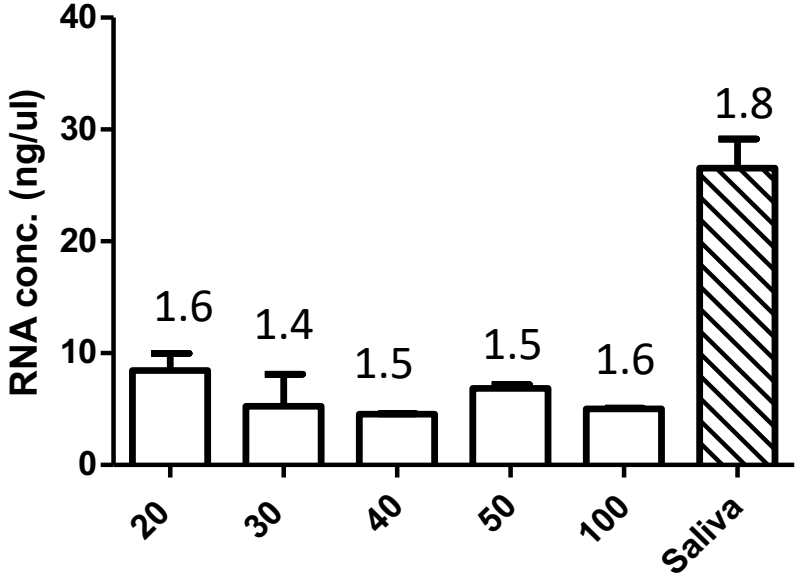


EBC breaths, n=6

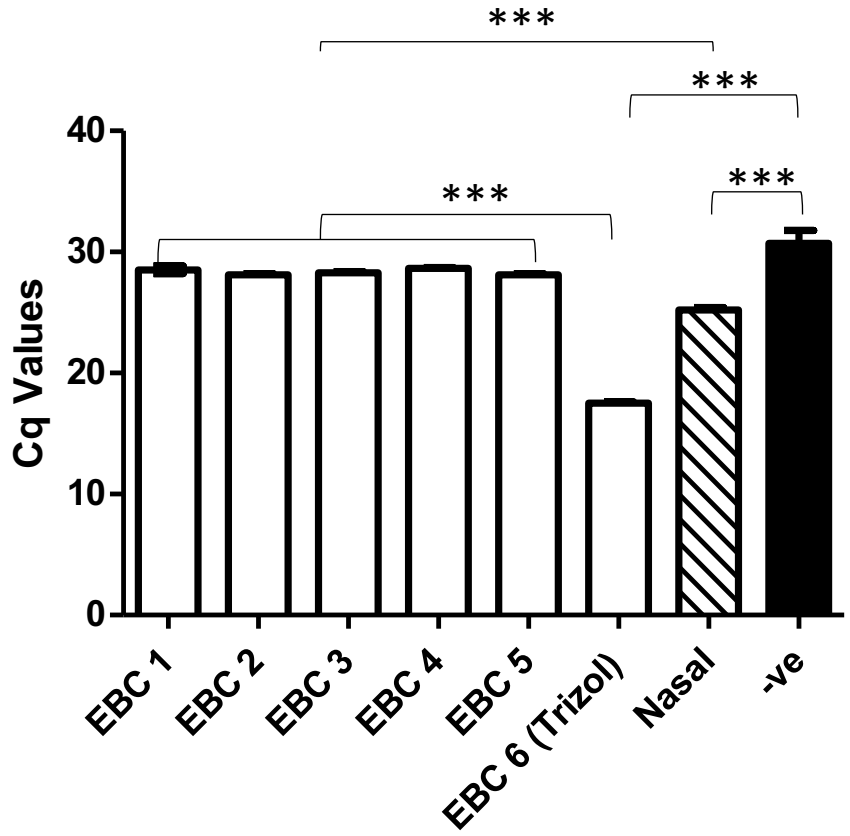
EBC 30-100 normalized to 20 breath sample volume, Trizol extraction

No statistically significant difference in concentration.

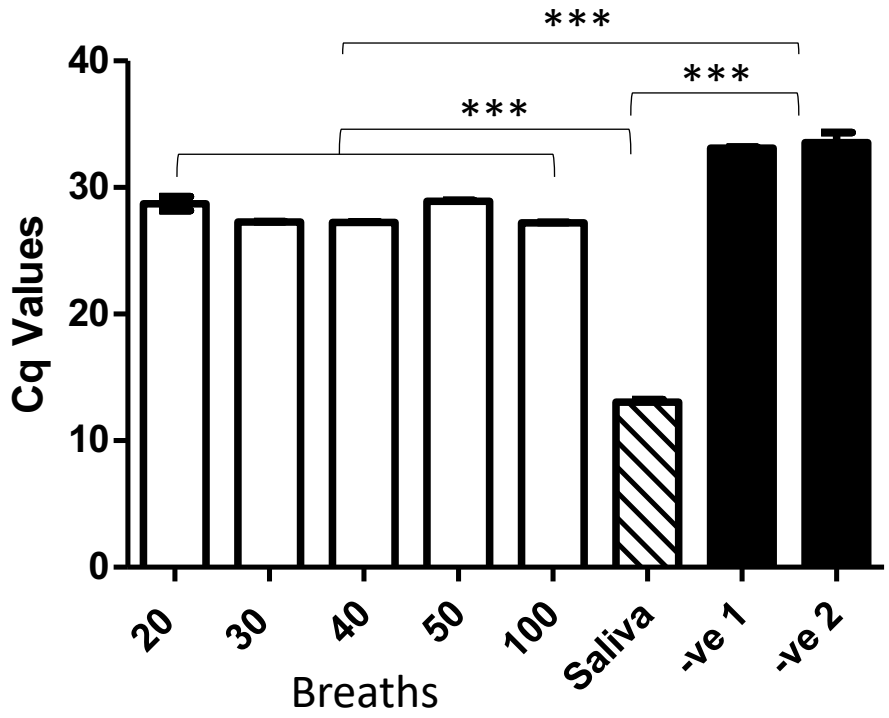
260/280 ratios reported per column

John Henderson
U. Northumbria

Prototype: 18S by PCR in EBC RNA



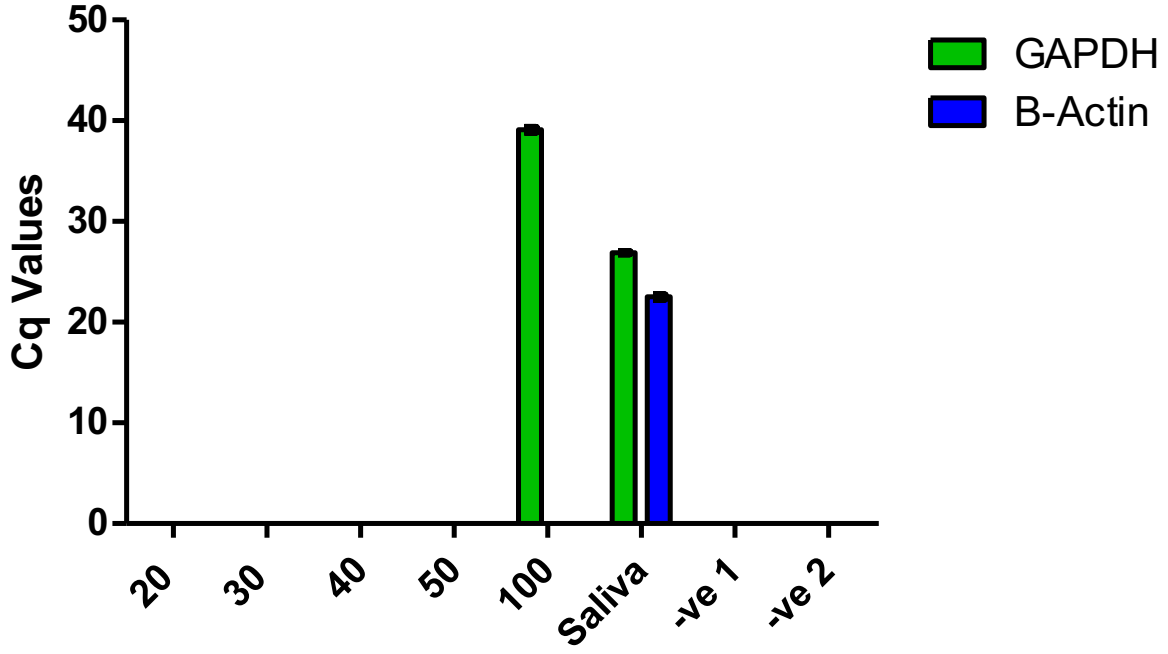
2 step SYBR Gold RT-qPCR (triplicate)
EBC1-5: RNeasy kit 20 breaths
EBC6: Trizol 30 min sample
Nasal swab.



2 step SYBR Gold RT-qPCR (triplicate)
-ve 1: No RT control
-ve 2: no cDNA
EBC 30-100 normalized to 20 breath sample volume
P<0.01 Saliva vs EBC, EBC vs –ve, Saliva vs –ve.

John Henderson
U. Northumbria

Prototype: Human RNA in EBC RNA



2 step SYBR Gold RT-qPCR (triplicate)
-ve 1: No RT control
-ve 2: no cDNA
EBC 30-100 normalized for EBC 20 sample volume

- EBC not classified as human tissue.
  - Human Tissue Act 2004.
  - Cells, DNA, or RNA.
  - EBC explicitly excluded.
- Early data: EBC is 18S+
  - Validation round under way with Taqman® assay.
  - ITS SEQ run planned (human vs fungal).

John Henderson
U. Northumbria

Prototype: Metabolomics in EBC

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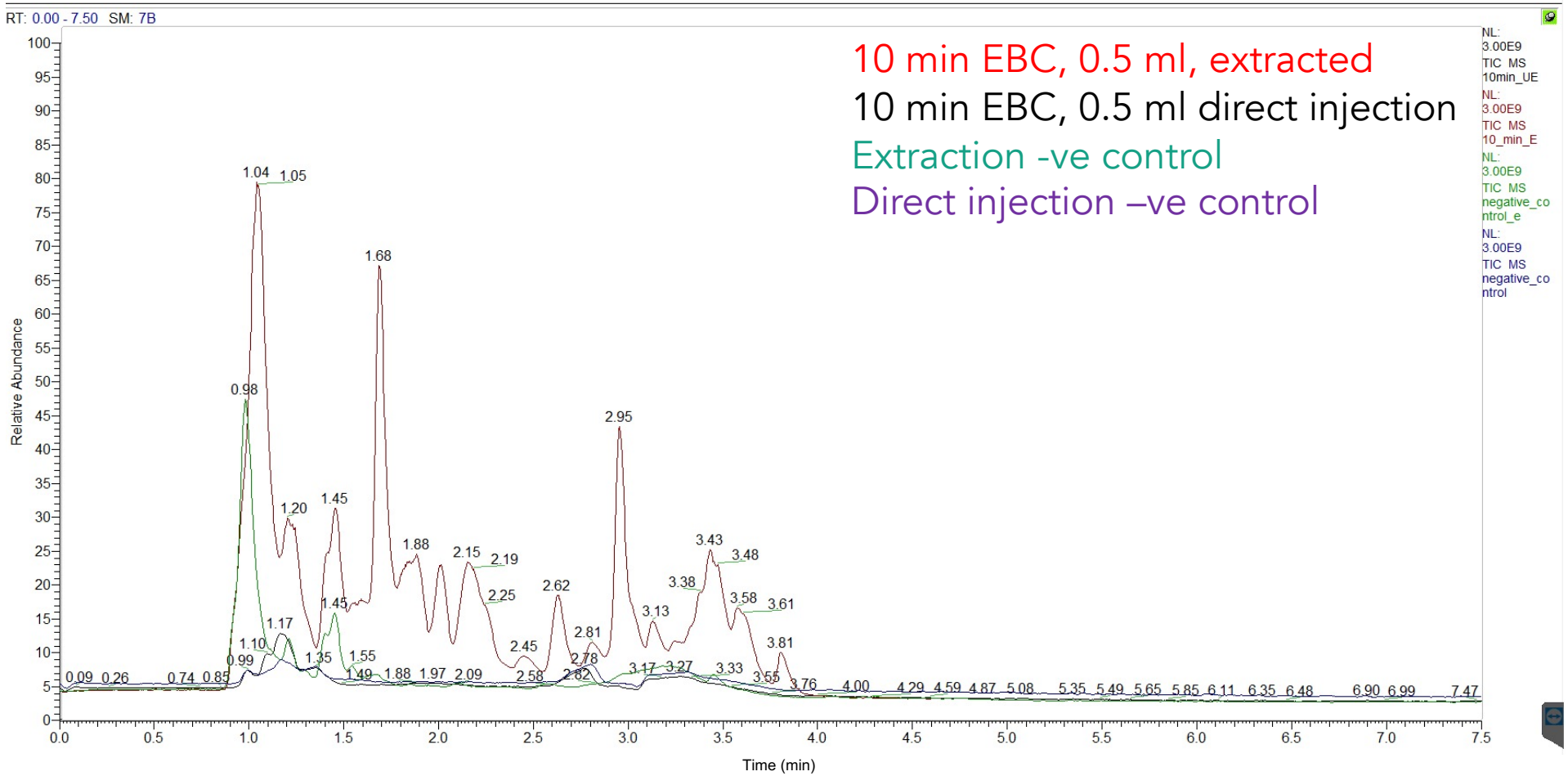

Metabolite profile in EBC after 5x concentration by lyophilization, and extraction

n=5

Data generated at the Northumbria University Metabolomics Core Service

John Henderson, William Cheung
U. Northumbria

Prototype: VOCs, C_{24}, lipids, meds, drugs

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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 at the Northumbria University Metabolomics Core Service

John Henderson, William Cheung
U. Northumbria

Prototype: 16S microbiomics detection of streptococcal mild cough.

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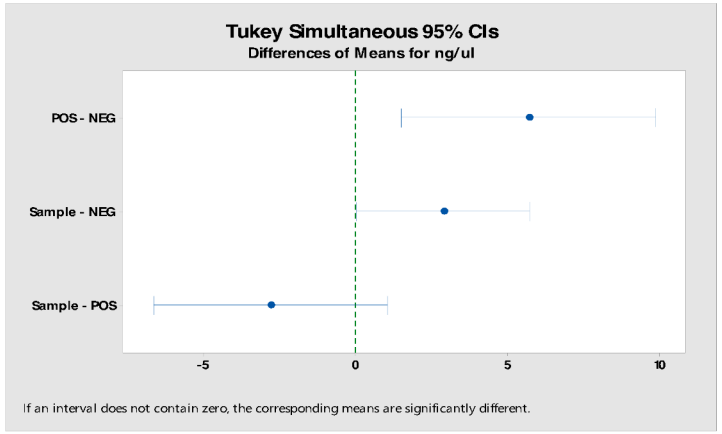

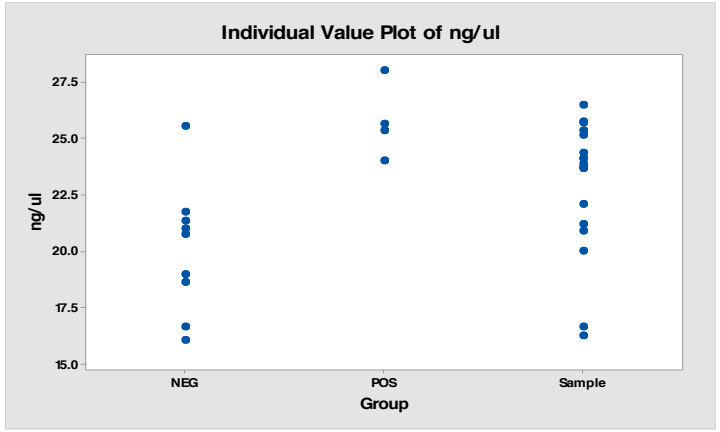

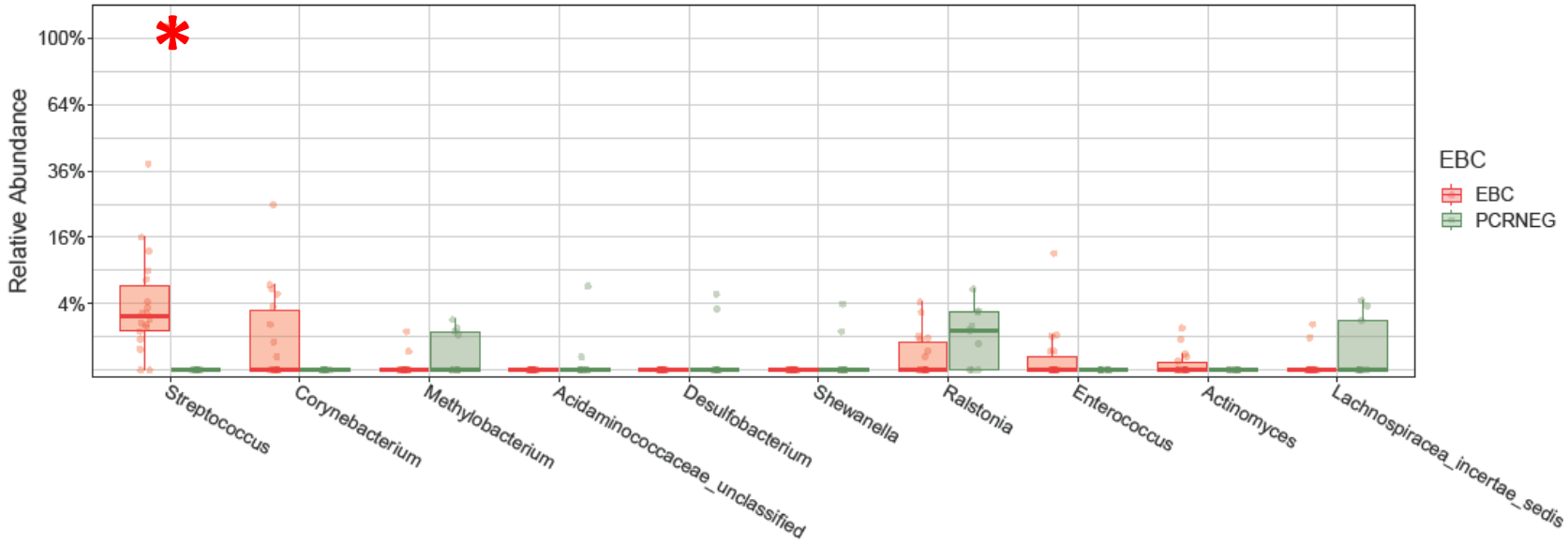

Higher DNA content vs background controls.

Detection of Streptococcus (BI FDR q = 0.019); amplicon generation w/out extraction.

Detailed work under way to optimize processes, polymerases, pipelines.

John Henderson
Andrew Nelson
Darren Smith
U. Northumbria

Prototype: As few as 1.7 aerosolized infectious virions detected per min.

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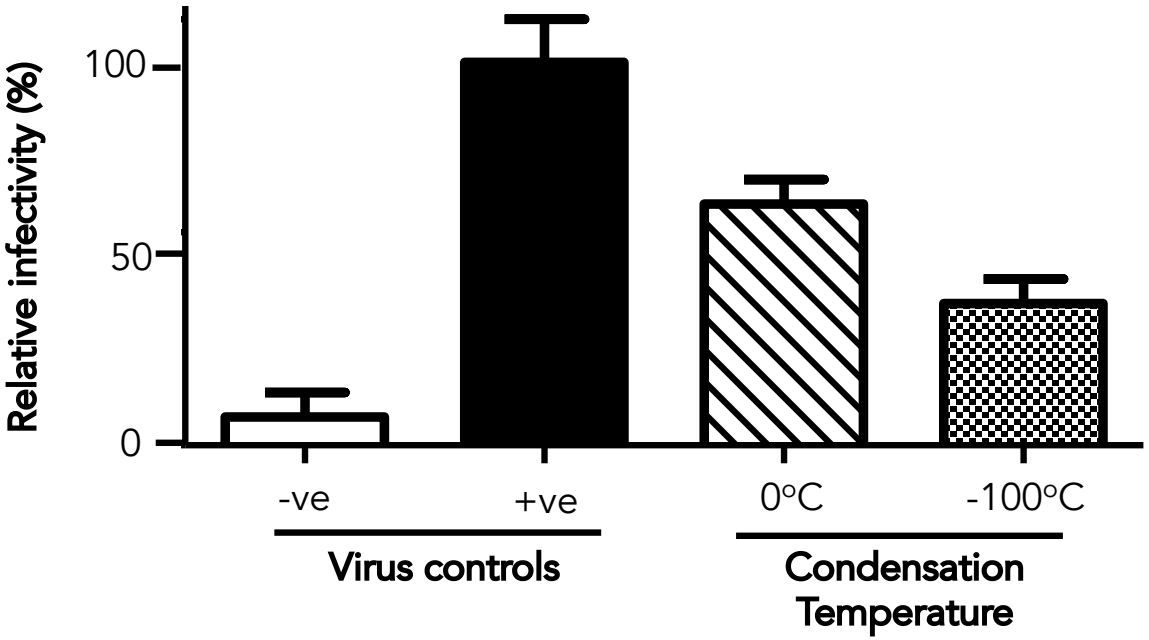
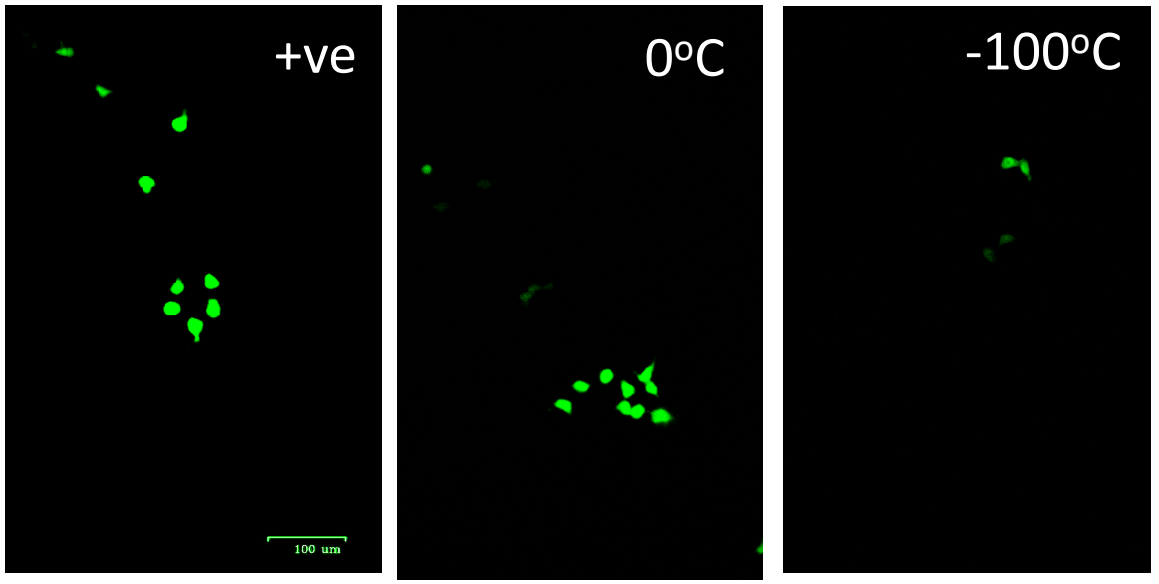
Efficient capture of aerosolized virus, halves infection risk.





GFP-expressing VSV-pseudotyped lentivirus nebulized using PARI TurboBoy SX and captured using PBM-HALE™ over 15 min). Condensates seeded on 10,000 HEK-293T's at 0.01 MOI. GFP expression measured at 72hrs by FACS, visualized by fluorescent microscopy. Bar = 100 um

John Henderson, Ed Wright
U. Northumbria, U. Surrey

External validation: Efficient Aerosolised SARS-CoV-2 Particle 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, diH_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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Pulmo BioMed

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- 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

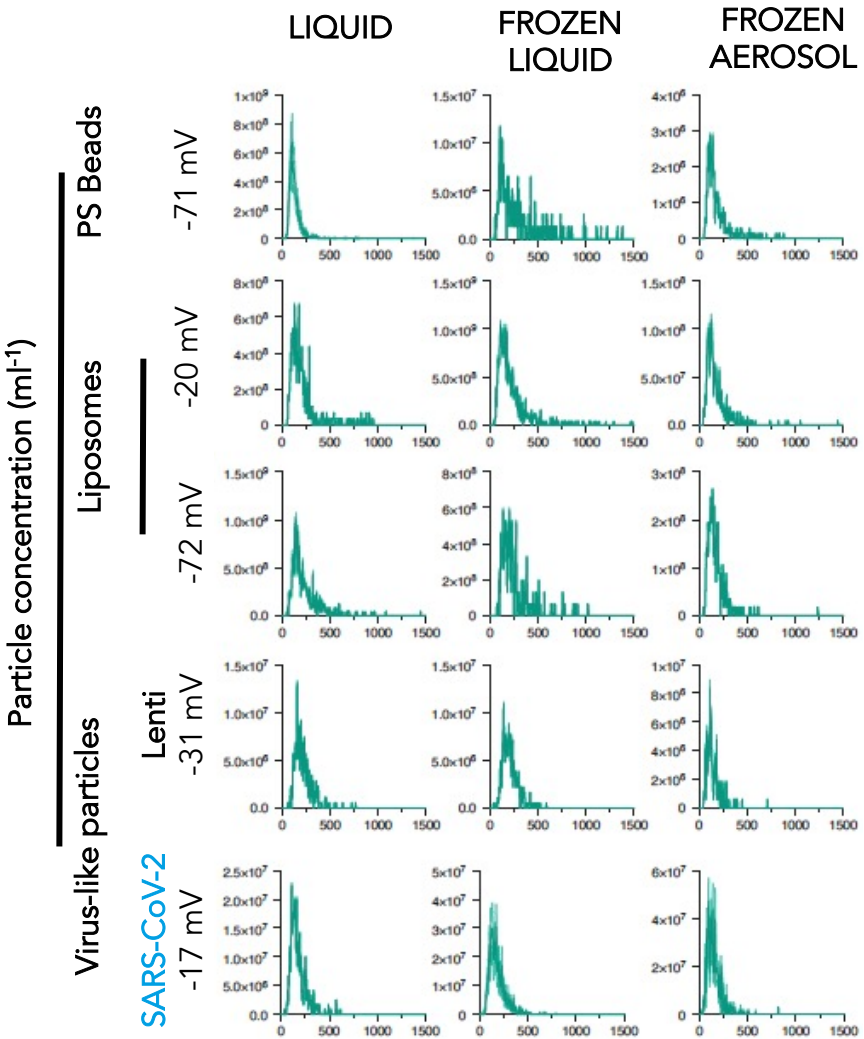
Sample analysis:

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

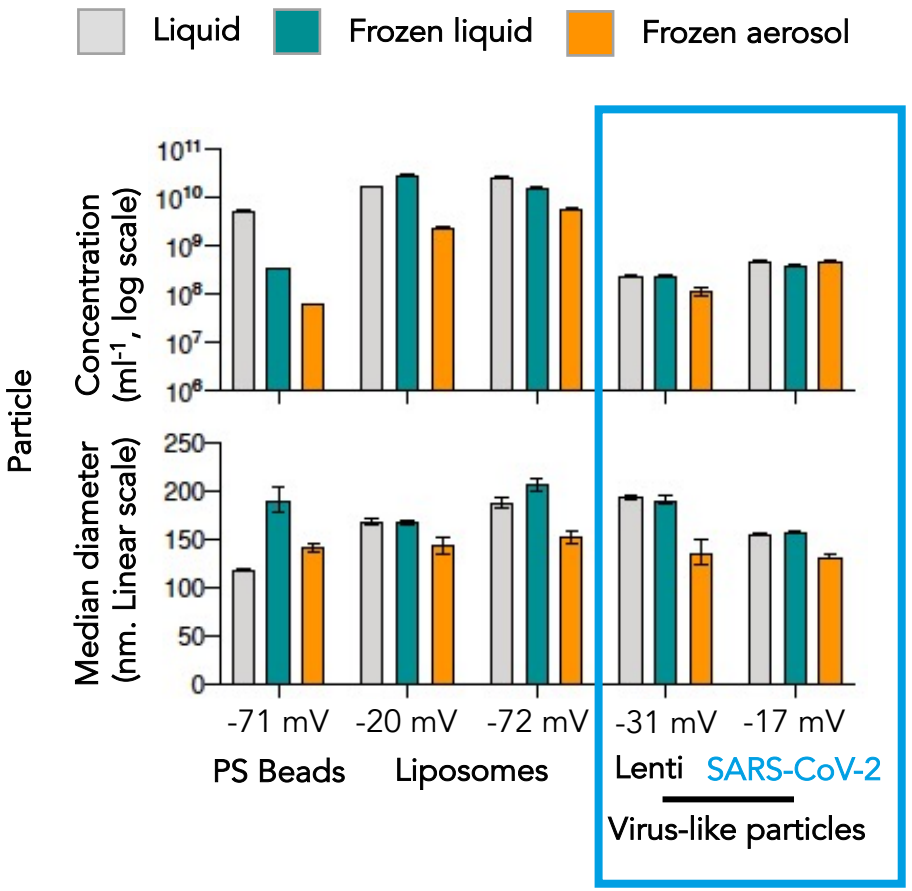
1. Based on fbioe.2020.00862 protocol

Rudiger Gross, Janis Mueller, Jan Munch
U. Ulm

External validation: Efficient Aerosolised SARS-CoV-2 Particle Capture



N=3, Shading covers SEM



Virually no loss of VLP size or structure

Aggregation with highly charged molecule

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

Rudiger Gross, Janis Mueller, Jan Munch
U. Ulm

Prototype: Clinical Pilot Update.

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NO FALSE POSITIVES:

- COVID19 patients (n=12).
  - Nasal swab negative.
  - Week 2-3 of symptoms, known nasal –ve period.
  - Antivirals / hydroxychloroquine.
  - (dyspnoea) 25-30 breaths/min.
- 5-20 min sampling.
- In COVID19 wards.
- Blinded analysis.

N=60 study ongoing

- Actively recruiting.
- Must be in 0-5 days from symptom onset
- Must be nasal positive by RT-PCR
- N=18 completed
- Interim data release: n=30
- Limited attendance at week 1 of symptoms slows recruitment.

Clinical data as of 5/05/21

We believe we can detect COVID-19 possibly with a 1 min sample

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- **Safely**: Kill the virus.
- **More reliably**: Larger sample than nasal swabs.
- **Simply**: With no skills needed: just breathe out.
- **Using mass screening**: by mass production of plastic.
- **Where patients are:** using *any* point of need testing system.
- **With current gold standard tests:**
  - e.g. Any laboratory RT-PCR instrument
  - e.g. Abbott ID NOW®: 5 min test or LFT/LFD
  - e.g. DeepVerge MicroTox BT: 4 sec test.

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

We believe we can detect COVID-19 possibly with a 1 min sample

Pulmo BioMed

- Confirm infectious virus load
  - By source of virus (oral, lung, nose)
  - By particle size (droplet, aerosol)
  - Optimise sampling maneuver.
- Determine the smallest sample amount needed for RT-PCR detection.
- Expand to pre/asymptomatic contacts / time course
- Deliver Emergency Use Authorisation (USA, UK).
- Produce >50,000 units.
- Supply at no profit basis under development funding.

ALL DEVICES PRODUCED THROUGH CHARITABLE/GOV FUNDING WILL BE MADE AVAILABLE AT COST ONLY FOR COVID19 PANDEMIC

How do I use the device?

Pulmo BioMed

1. Device use SOP: https://youtu.be/h6tLt9u-rWU

2. Lay explanation of use: https://youtu.be/TkQEj-KN_os

Pulmo BioMed

Your Breath: Your Health™

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This slide deck is live and will be updated on an ASAP basis.

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