

Infectious & genetic viral load in exhaled breath aerosols.

MicroSoc SARS-CoV-2 COVID-19 Workshop July 2020

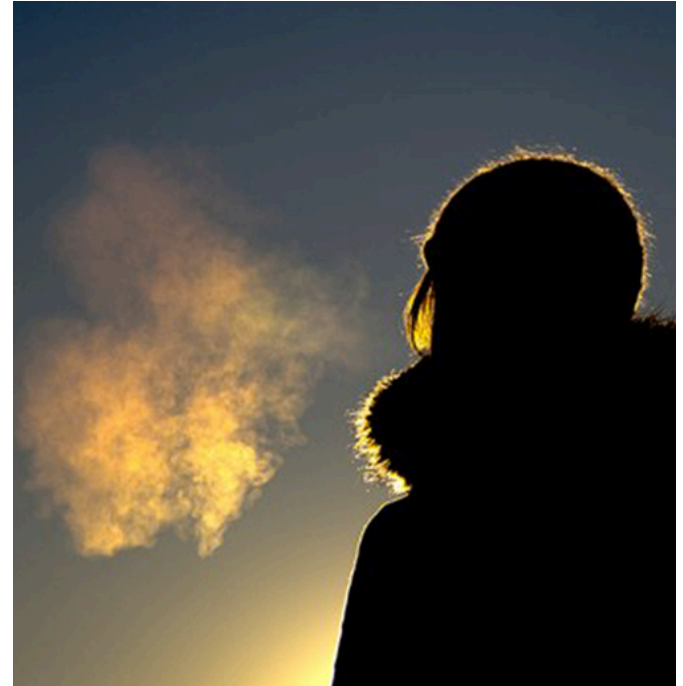
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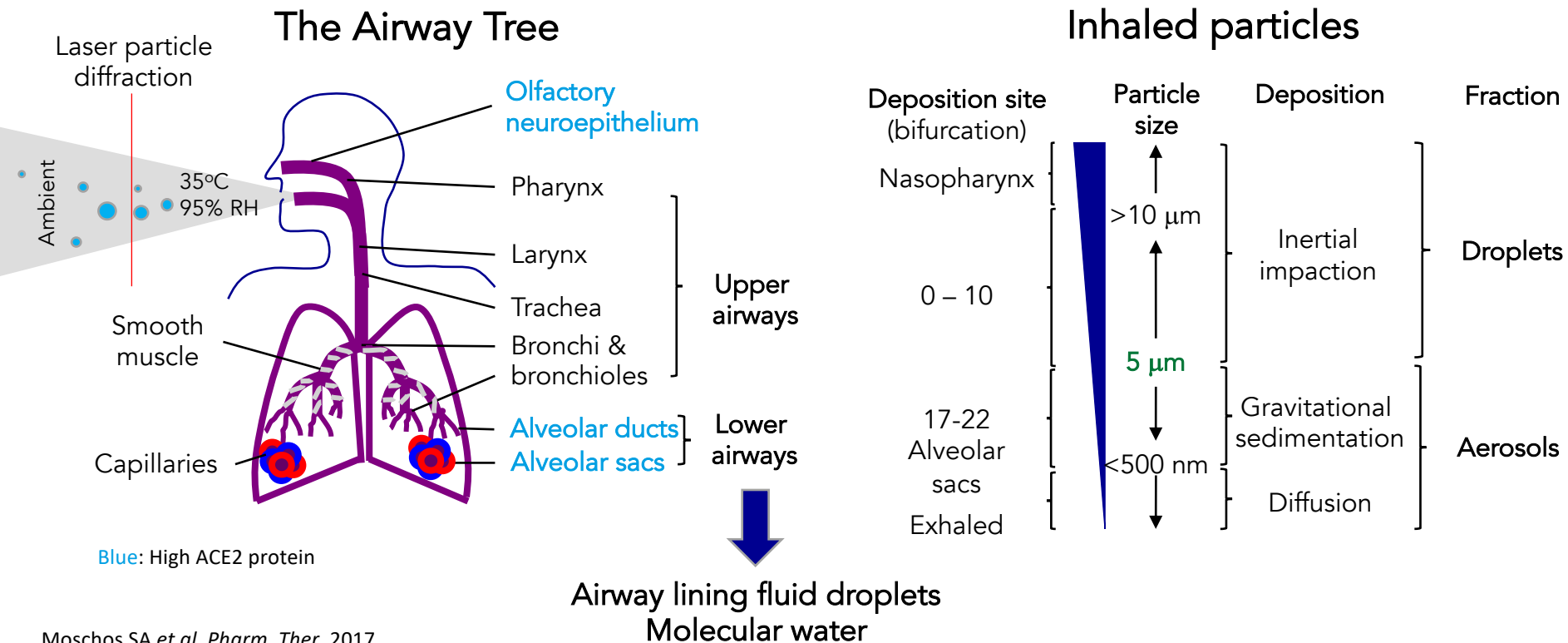
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Overview

- Exhaled Breath and SARS-CoV-2.
 - Background.
 - Hypothesis.
 - The challenge.
- The PBM-xHALE™ approach.
 - Platform.
 - Supporting data.
- Clinical progress update.



Exhaled breath: origin, content, COVID19.



Coronavirus 19 (COVID-19) in breath: a Hypothesis

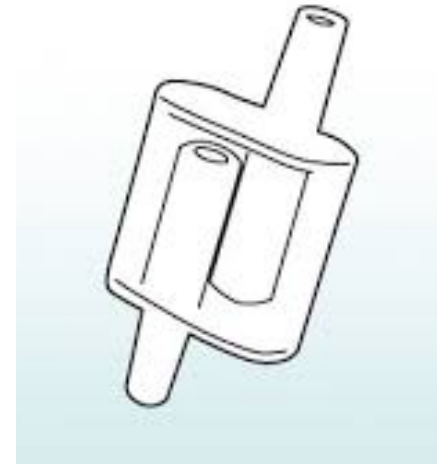
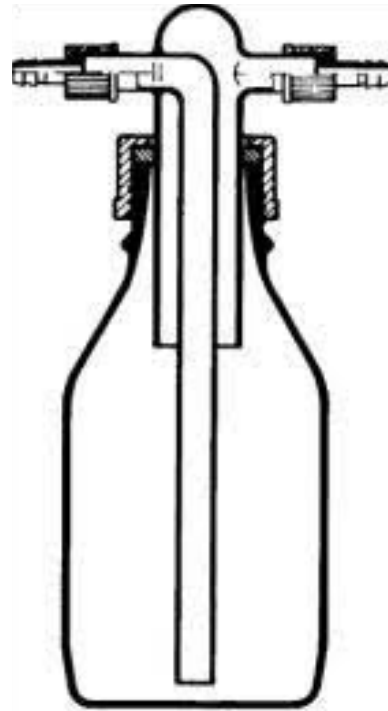
- Disease is a function of amount of virus reaching the lower lung.
- Deep lung accessed mainly by breath aerosols.

We need to test breath aerosols for:

- *The amount of virus present (genomes).*
- *Infectivity (viruses).*

Exhaled breath condensates (EBC)

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



Alco-pro saliva trap

Exhaled breath condensates (EBC)

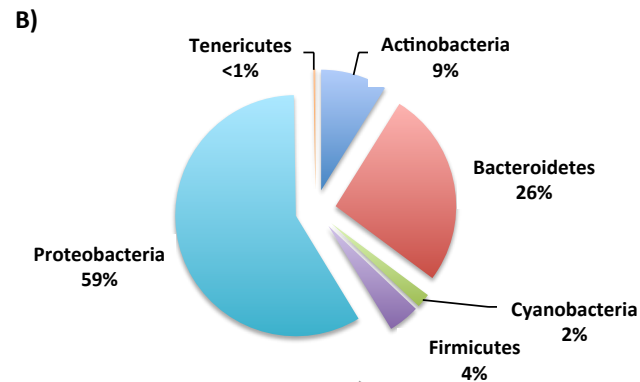
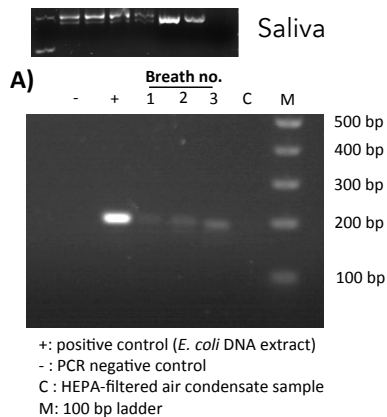
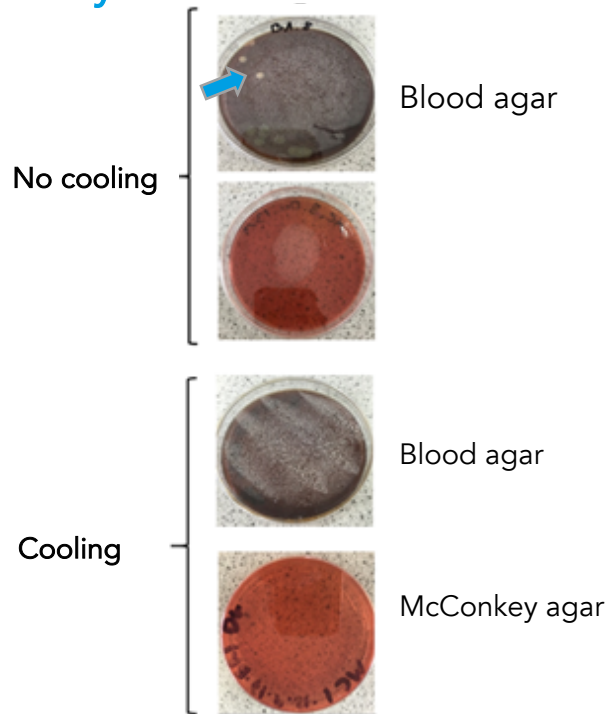
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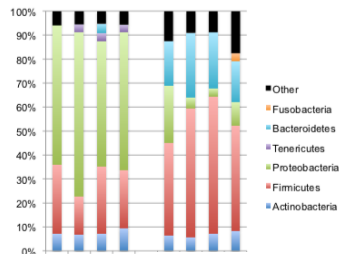
Preliminary data: bacteria detection

Bacteria & fungi die by dry ice condensation

NAATs work with 1-3 breaths (bacteria)



EBC is distinct to 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:

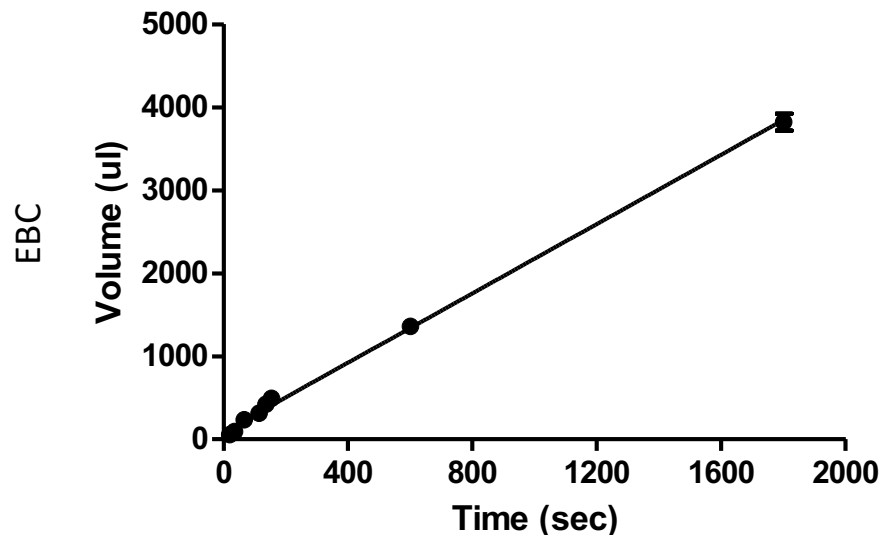
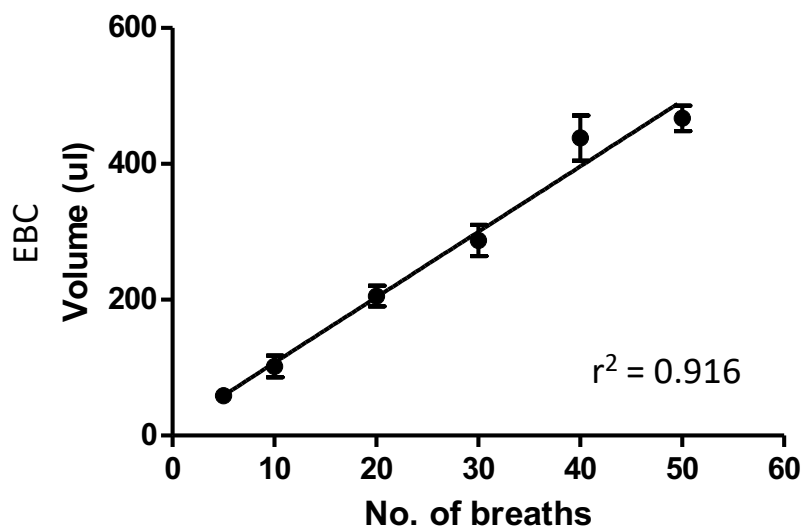
- Volatiles and
- Proteins.
- DNA.
- RNA.
- Lipids.
- Medications.

Solves key problems:

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

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

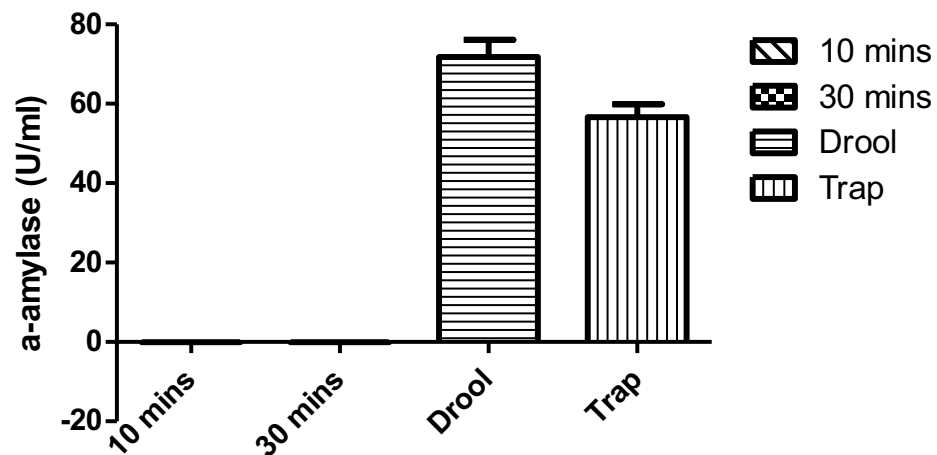
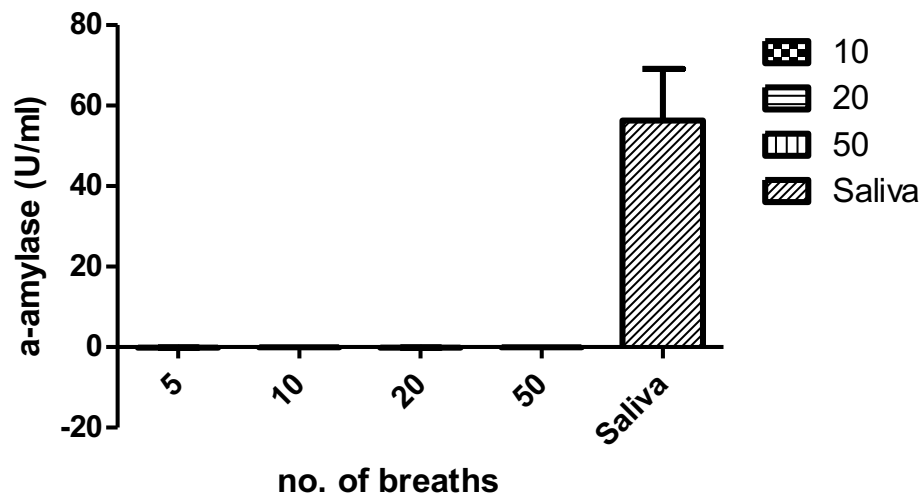
Prototype: highly consistent sampling



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

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

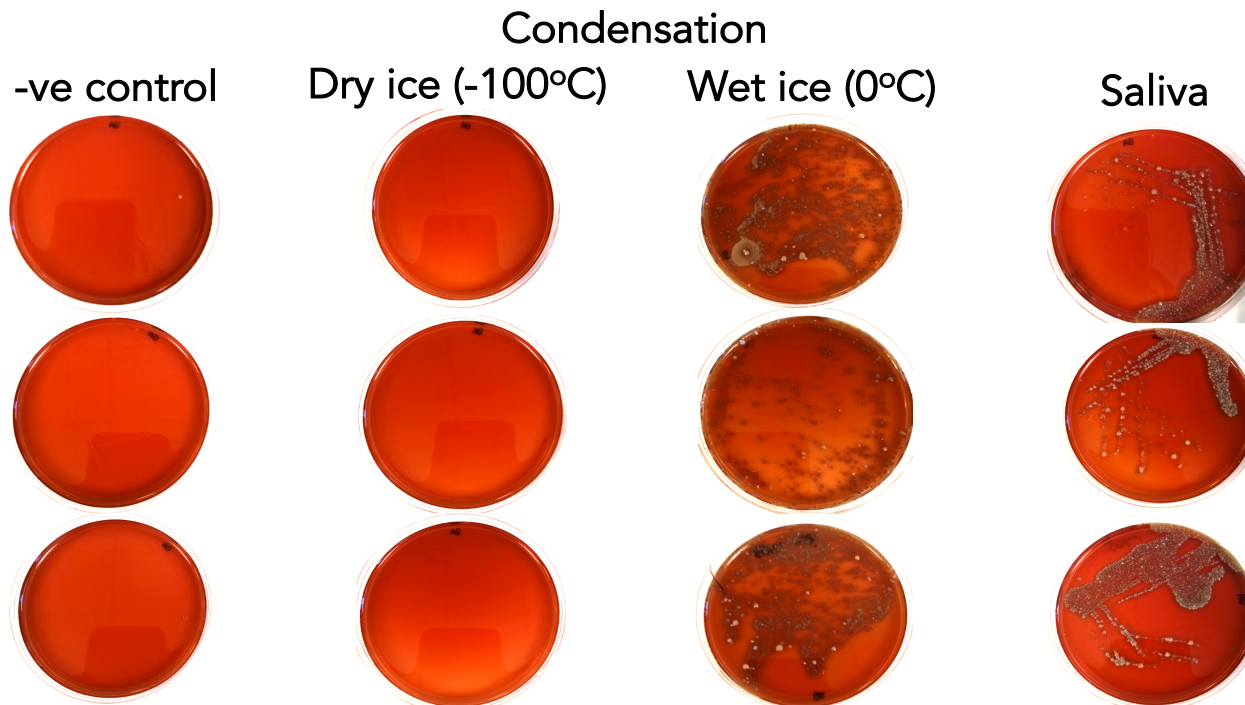
Prototype: no salivary contamination



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

n = 5.

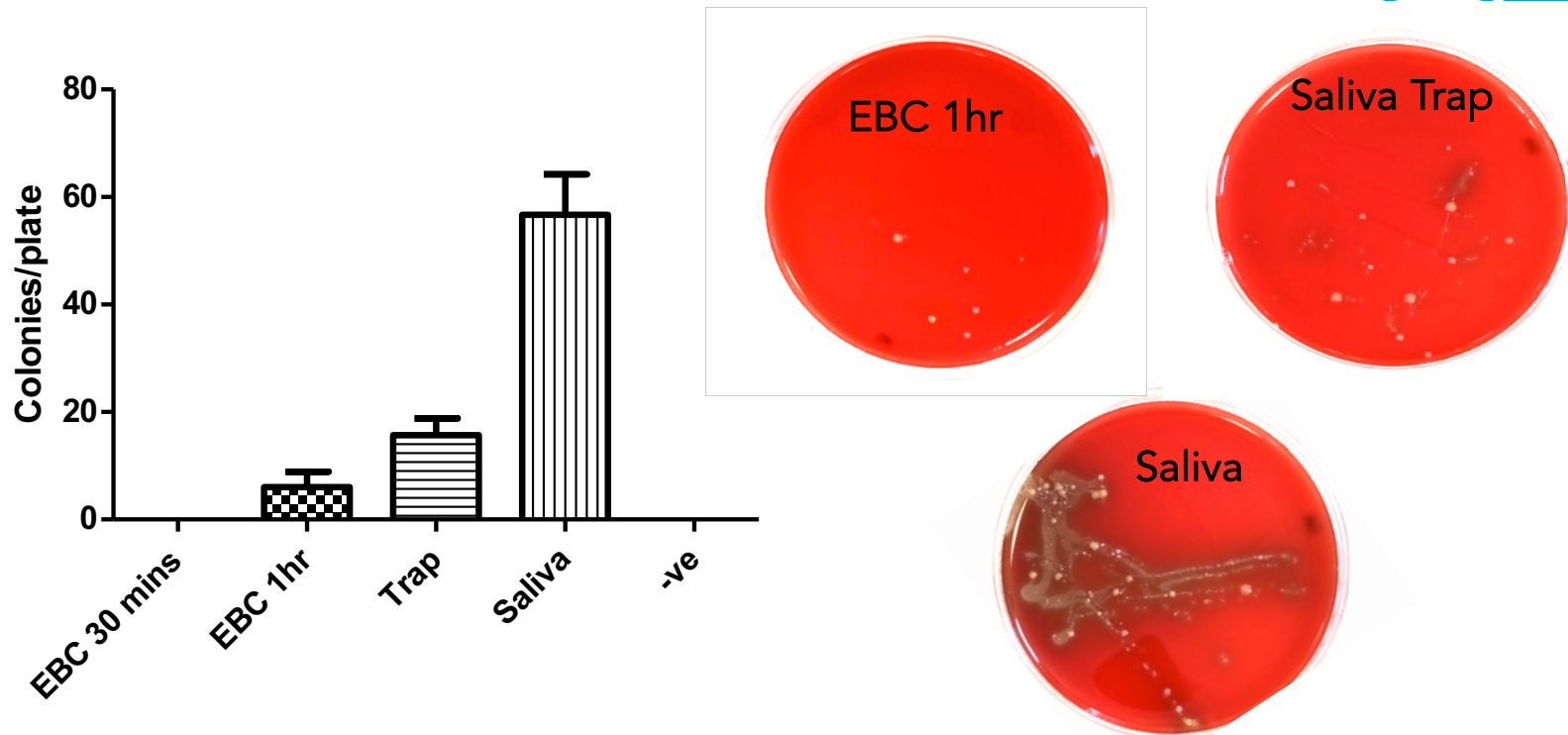
Prototype: dry ice eliminates viability



2 min sampling period

n = 5, blood agar

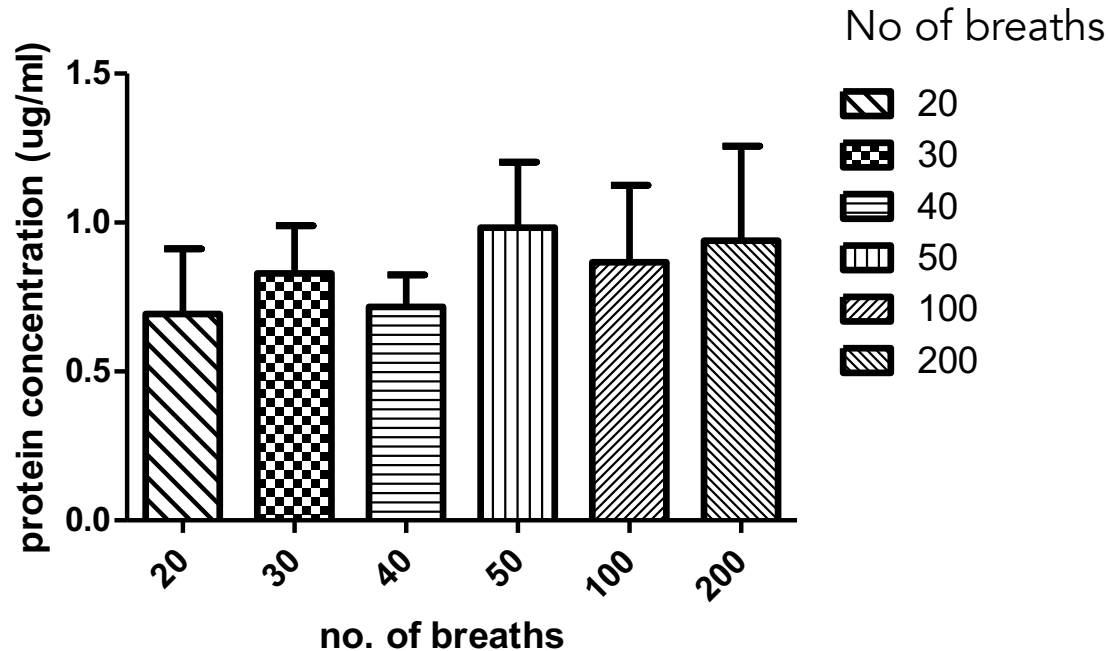
Prototype: dry ice eliminates viability



Cooling efficiency lost after ~40 min continuous sampling.

n = 3.

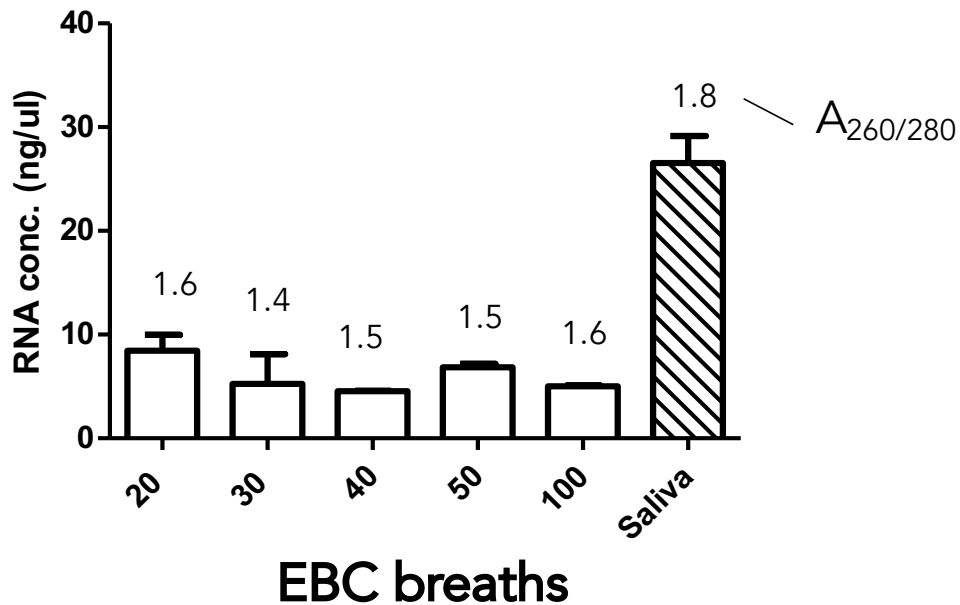
Prototype: consistent [protein] in EBC



Requires 5x concentration by lyophilization

n=5

Prototype: consistent [RNA] in EBC

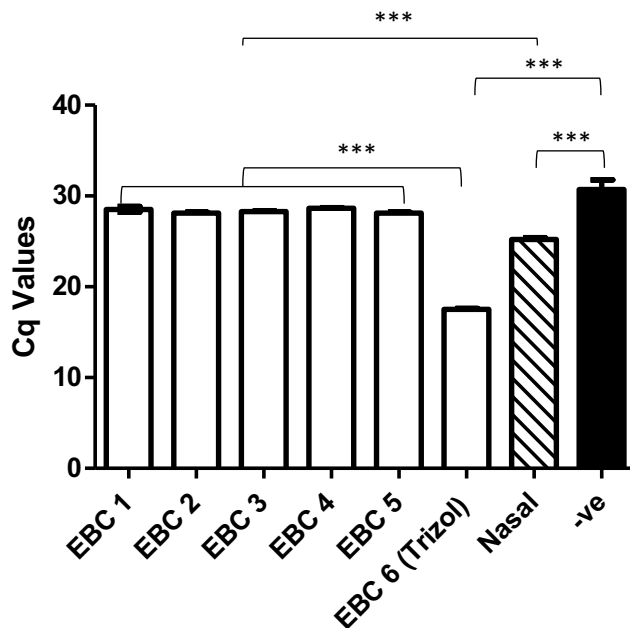


Increased EBC sampling does not increase [RNA].

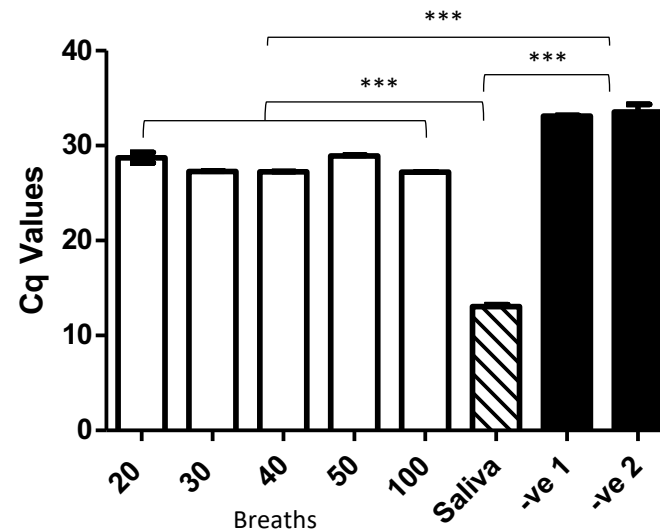
EBC volume extracted by Trizol matched with volume obtained after 20 breaths.

n=6

Prototype: 18S in EBC RNA

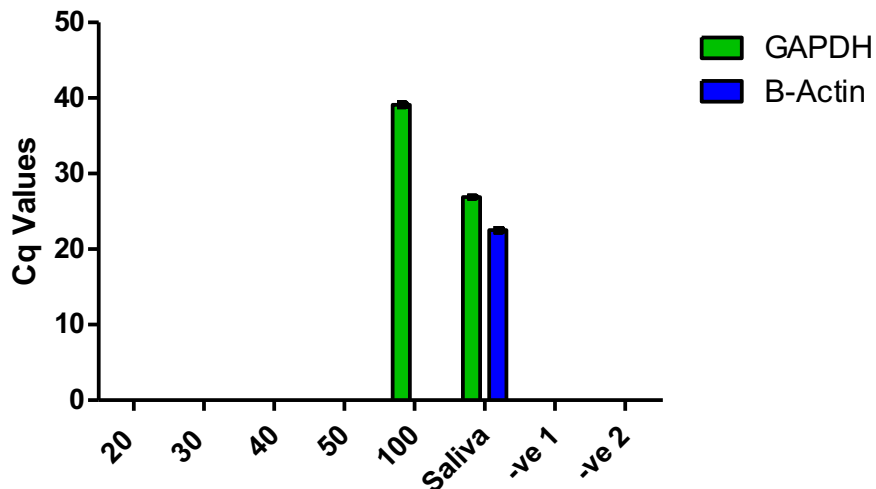


2 step SYBR Gold RT-qPCR (triplicate)
 EBC1-5: RNeasy kit 20 breaths
 EBC6: Trizol 30 min sample
 Nasal = swab.



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

Prototype: GAPDH & β -actin in EBC RNA



2 step SYBR Gold RT-qPCR (triplicate)

-ve 1: No RT control

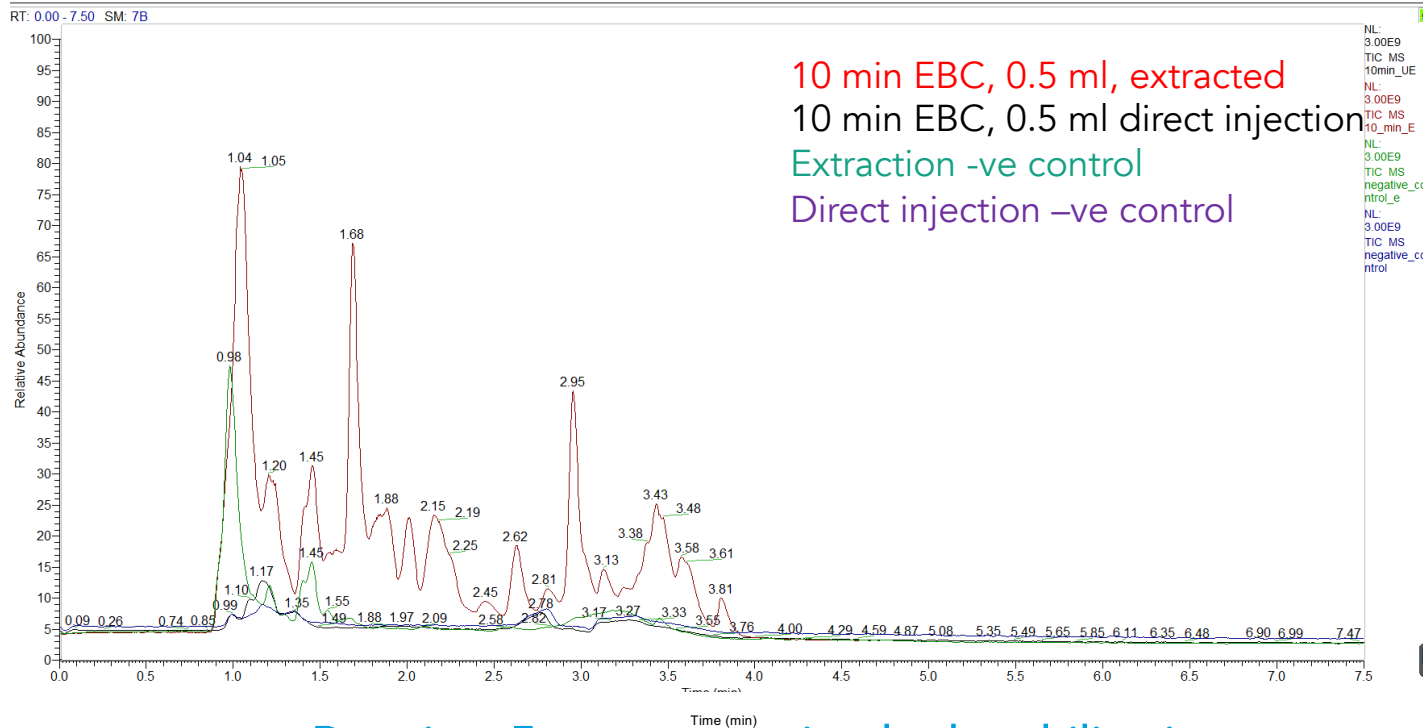
-ve 2: no cDNA

EBC volume normalized to 20 breaths

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

n=5

Prototype: metabolomics in EBC



Requires 5x concentration by lyophilization

n=5

Prototype: metabolomics in EBC

Compound	RMM (g/mol)	RT [min]	Relative ion abundance
1-hexadecyl-glycero-3-phosphate monoacylglyceride	396.3	1.002	810,094
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

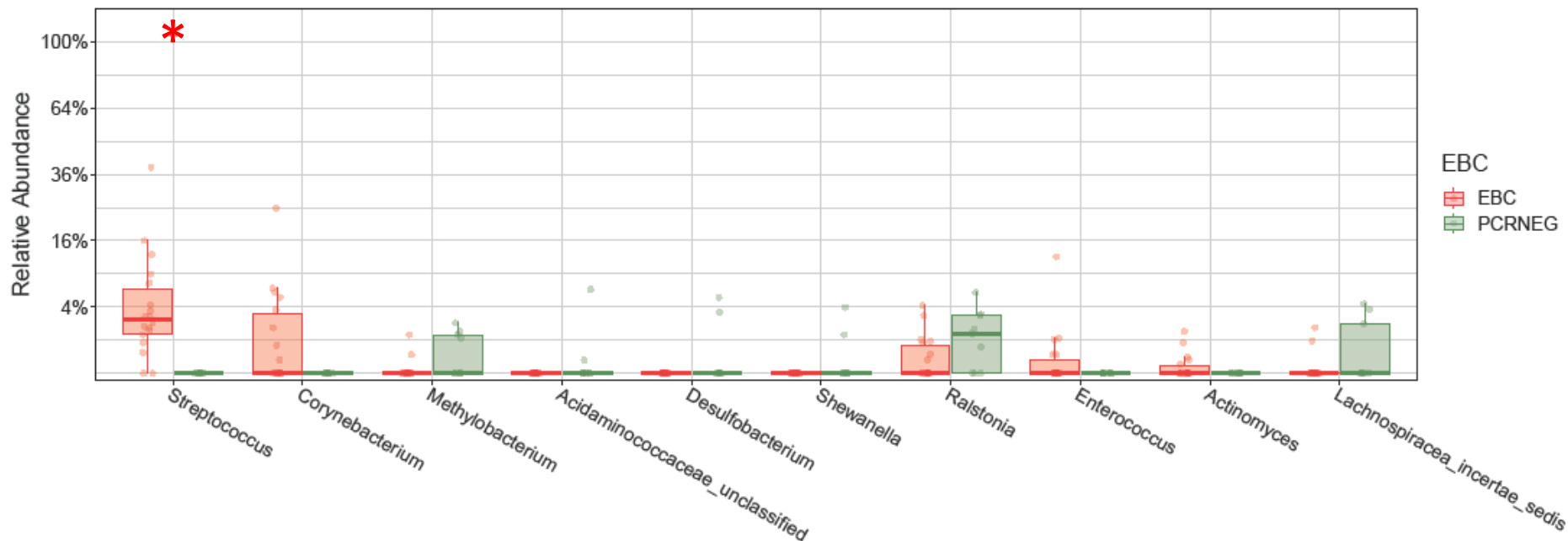
Compounds detected by MS1:

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

Additionally:

- 20 multiple HDBM hits.
- 104 novel compounds.

Prototype: 16S microbiomics of EBC

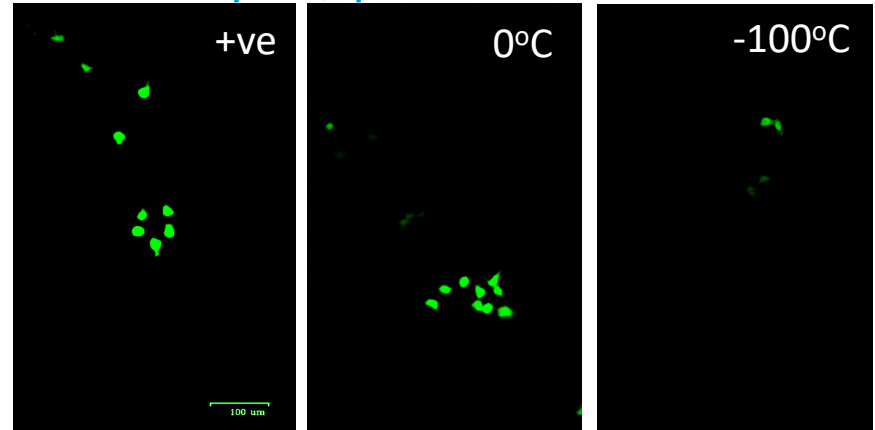
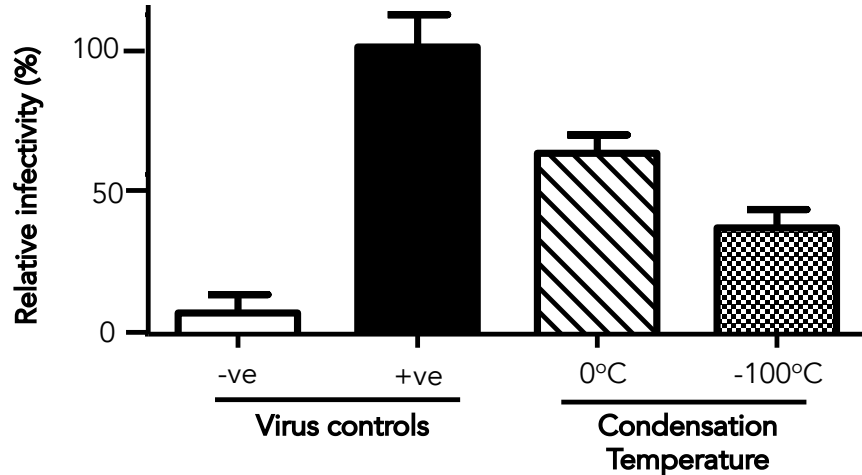


Higher DNA content vs background / kit controls

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

Prototype: detection of virus aerosol

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 µm.

Prototype: COVID-19 Pilot data.

- <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).

30-60 sec seems adequate.

We believe we can detect SARS-CoV-2 in tidal breath.

- Fast: <1 min sample.
- Simple: just breathe.
- >2x safer: process halves infectivity.
- More reliably: Larger sample than 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[®]).

Next steps



Research

- No false negatives vs swab:
 - N=100 study.
 - EUA.
- Detect virus by LFT.
- Eliminate cold chain.
- On-board analytical capability.

Development

- Spinout incorporated, seed round.
- Complete series A.
- MHRA/FDA/WHO.
- Mass production, distribution.

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

Acknowledgements



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Mr. Jonathan Brooks (COO).



Innovate UK



Consultancy disclosures:

