SARS-CoV-2 load in exhaled end-tidal breath fine aerosol condensates among acutely symptomatic COVID-19 cases

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Introduction

What is the source of SARS-CoV-2 virions in exhaled breath fine aerosols?

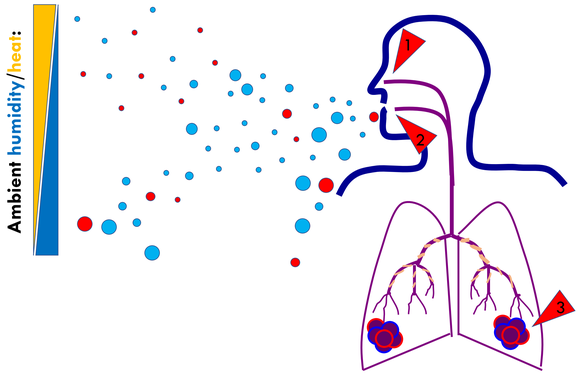

Figure 1: The source of SARS-CoV-2-laden aerosols in breath remains unclear . SARS-CoV-2 (red circles) has been reported mainly in fine aerosols but also in large droplets in exhaled breath. It is also found in substantial quantities in nasopharyngeal swabs (1), saliva (2), and bronchoalveolar lavage (3; red triangles). The source of wet and dry aerosol particles in breath can be the nasal mucosa, the mouth, as well as the large and small airways, and the alveoli. Large droplets arise from the upper respiratory tract and mouth, and fine aerosols from the distal lung. However, upon expiration, aerosols rapidly swell or shrink depending on ambient conditions (heat, humidity). Most exhaled breath samplers either don't separate aerosol fractions or allow for aerosol size evolution.

SARS-CoV-2 has been detected in:

- Environmental aerosols
- Exhaled breath
- Nasopharyngeal swabs
- Saliva
- Bronchoalveolar lavage

Most breath sampling approaches do not:

- Separate aerosols by size
- Eliminate oral large droplets (saliva)
- Prevent aerosol size evolution (fig 1)
- Prevent fomite, salivary, manual or environmental sample contamination
- Feature stable sample capture conditions (temp, pressure)

Hypothesis

Hypothesis: Exhaled breath condensate capture after separating large droplets by inertial impaction close to the mouth can quantify viral load in distal lung fine aerosols.

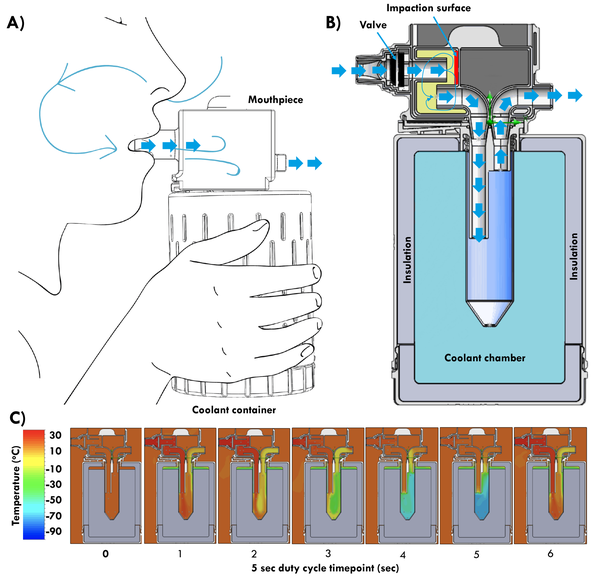

Figure 2: The PBM-HALE™ device captures contaminant-free breath fine aerosols from the distal lung. (A) Schematic of a patient inhaling via the nose and exhaling (blue thick arrows) via the mouthpiece of the device. **(B)** The mouthpiece features a one-way (expiration only) valve preventing inhalation through the device. Within 4 cm from the mouthpiece entry point is an inertial impaction surface (red line) that captures large droplets within the mouthpiece space (yellow). Turbulent flow (thin blue lines) proceeds inside the mouthpiece around the pipes, into the 90º downward elbow pipe, and into a 50 mL vial (blue). The vial is immersed in coolant (cyan), typically dry ice (-78.5°C) inside an insulated, locked coolant container. **(C)** Computational flow dynamic model of a 5 sec breathing cycle showing condensation occurs during the static phase in the device when the patient inhales nasally. Aerosol condensation on the internal surface of the vial happens rapidly during the inhalation phase of the breathing cycle capturing the terminal 48 mL of expiration. The vial is removed at the end of sampling in a sealed configuration preventing manual/ambient sample contamination.

PBM-HALE™ features

- Proximal inertial large droplet removal (saliva; Fig 2)
- High thermal capacity coolant (stable temp.)
- End-cycle (distal lung) fine aerosol condensation
- Sealed sample vial prevents fine aerosol contamination

Methods

Sites & ethics

- Northumbria University, Newcastle Upon Tyne, UK (Application no. 43341 approved by the Department of Applied Sciences Subcommittee of University Research Ethics Committee)
- Athens General Hospital "Evangelismos", Athens, and University General Hospital of Herakleion, Crete, Greece (Protocol no. 280/24-4-2020 approved by the Scientific Committee of the General Hospital "Evangelismos".)
- Centre for Advanced and Innovative Therapies, Federal University of Minas Gerais, Minas Gerais, Brazil (Approval no. 54358021.1.0000.5149 approved by the Institutional Review Board of the Federal University of Minas Gerais

Participant recruitment

- 18-70, informed consent
- Acutely symptomatic (days 0-5 from symptom onset)
- LFT positive nasopharyngeal swab
- Not on antiviral treatment

Clinical settings

Greece:
- COVID-19 wards or 'red zone' screening rooms
- June 2020 to June 2022
- No HEPA filtration
- No mechanical ventilation

Brazil:
- Suburban primary care centre
- March 2022 – June 2022
- Naturally ventilated (high airflow) room in converted container

Sampling procedures

Greece:
- Nasopharyngeal swab
- Tidal breathing, <30 min

Brazil:
- Nasopharyngeal swab
- Tidal breathing, <30 min
- Forced expiration, <15 min

Analysis

Greece:
- Up to 1 ml of breath fine aerosol sample extracted
- QIAsymphony DSP Midi kit automated extraction (60 µL)
- VIASURE SARS-CoV-2 ORF1ab and N, internal assay control; 1replicate on 5 µl of extract

Brazil:
- Entire breath fine aerosol sample volume extracted
- Manual RNA extraction (70 µL)
- US CDC SARS-CoV-2 multiplex assay (N1, N2), and 18S rRNA; technical triplicates on 10 µl of extract each.

Results

High viral loads in fine aerosols under forced expiration

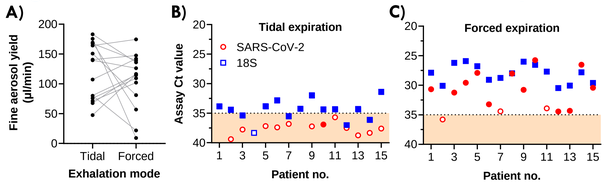

Figure 4: Forced expiration results in 100% detection of SARS-CoV-2 in distal lung fine aerosols. (A) Fine aerosol yield per min exhalation is not affected by exhalation mode (Wilcoxon matched pairs, signed-rank test; p = 0.6387). **(B)** SARS-CoV-2 was not detected in tidal expiration fine aerosols by single replicate VIASURE RT-PCR (n = 30; Greece) despite internal control amplification. Only a single positive (filled data points) was detected by CDC RT-PCR (n = 15; Brazil; 3/15 negative; 11/15 inconclusive (hollow data points). **(C)** Singing loudly "happy birthday" resulted in 12/15 positive and 3/15 inconclusive detections of SARS-CoV-2 by technical triplicate CDC RT-PCR on fine aerosols (n = 15; Brazil). 18S Ct values are correlated to SARS-CoV-2 Ct values by non-parametric Spearman correlation (p = 0.0213). Inconclusive detections defined as <3/3 replicate amplifications in at least one of the two N assays. The CDC SARS-CoV-2 assay limit of quantification (Ct = 35) is shown using a dotted line and beige band. Plate positive and negative controls performed as expected.

No salivary contamination of fine aerosols

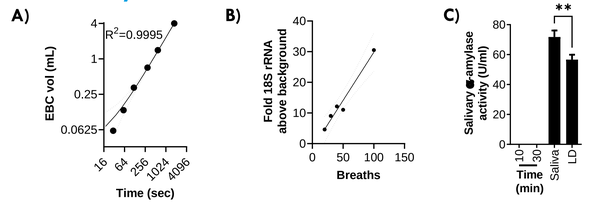

Figure 3: The PBM-HALE™ device performance in healthy volunteers. (A) Fine aerosol sample volume as a $\log_2$-$\log_2$ function of sampling time in a healthy adult volunteer. **(B)** Total RNA levels as a function of sampling time, as measured by pan-eukaryotic 18S rRNA RT-PCR on TRIzol-extracted fine aerosol RNA; no human b actin, GAPDH, or RNase P (RP) was detected. **(C)** Salivary alpha amylase activity (Salimetrics) in fine aerosols after 10 or 30 mins of sampling vs paired salivary or large droplet (LD) fraction activity; data representative of n>500 separate tests to date showing no amylase in fine aerosols, indicating dilution (if any) of saliva > 1:1,750x).

No ambient viral RNA contamination of fine aerosols

- N=2 convalescent cases, negative nasopharyngeal RT-PCR
- N=10 cases at >2 weeks from symptom onset
- All SARS-CoV-2 negative (no inconclusive results)

Acute COVID-19 patient characteristics

Greece:
- N = 30; 47.2 ± 17.1 yo; 50% ♀; BMI 25.2 ± 8.12
- Nasopharyngeal Ct = 21.2 ± 5.43; days from symptom onset: 3.17 ± 1.09

Brazil:
- N = 15; 48.2 ± 11.5 yo; 73.3% ♀, BMI 27.9 ± 5.8.
- Nasopharyngeal Ct = 22.8 ± 2.71; days from symptom onset: 3.52 ± 1.06

High SARS-CoV-2 loads are found in distal lung fine aerosols produced during forced expiration.

- PBM-HALE™ captures saliva-free, fine aerosols from the distal lung
- Fine aerosols from tidal breath have negligible viral load
- Forced expiration allows for 100% detection and presents significant airborne transmission risk

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