The SARS-CoV-2 Viral Load in COVID-19 Patients is Lower on Face Mask Filters than on Nasopharyngeal Swabs

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1. Background and Objectives

The COVID-19 pandemic has sparked an enormous amount of new research, with over 160,000 papers mentioning it currently listed in PubMed. Notably, it has led to increased interest in breath sampling as a means to detect and diagnose illnesses, and various approaches have been explored to use either exhaled breath condensate (EBC), exhaled breath aerosols (EBAs), or volatile organic compounds (VOCs) to identify infection.

EBAs consist of respiratory droplets of a range of sizes (**Figure 1A**) which are a key vehicle for SARS-CoV-2 transmission. The ability to capture EBAs and analyze them to detect virus particles represents a significant opportunity to improve virus detection using non-invasive methods.

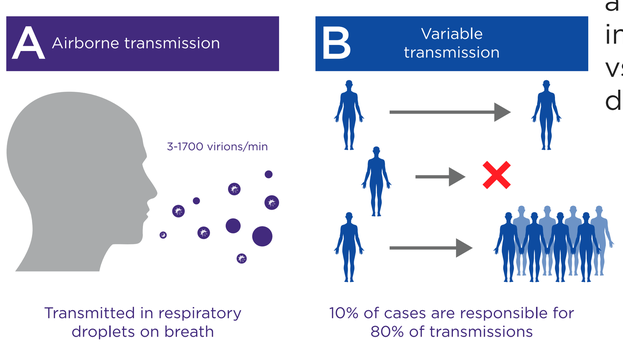


Figure 1: (A) COVID-19 transmission is airborne through exhaled respiratory droplets. (B) Transmission varies between cases with a small number responsible for the majority of new infections.

Currently, nasophayngeal swabs are the most widely used means to detect SARS-CoV-2 infections both via PCR and for lateral flow tests. NPS is effective but the sampling method is invasive and unpleasant. Not all who are infected with COVID-19 will spread the disease (**Figure 1B**). and more acessible and acceptable methods may be needed to bring the pandemic under control.

EBA can be captured from breath during regular use of a mask by integrating a simple filter that can then be tested for the presence of viral particles (**Figure 2**).

Aim: Assess the sensitivity of EBA as a means to detect respiratory infections. Then test the utility of EBA vs. nasopharyngeal swabs (NPS) as diagnostic tests for COVID-19

2. Mask Filters for EBA Capture

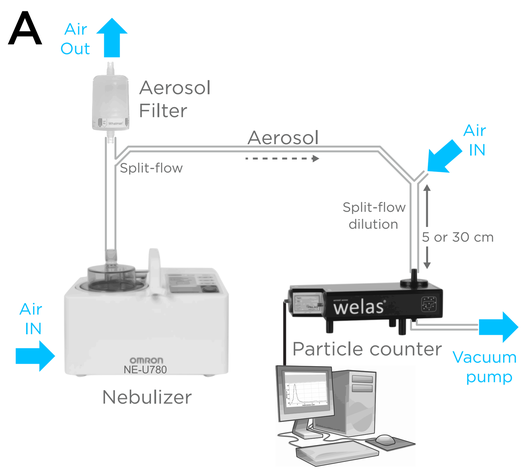
Figure 2: The mask and filter set-up used for EBA collection and virus detection. The masks used were adapted from the ReCIVA® Breath Sampler and include an integrated electrostatic filter. In principle, similar filters could be used with other types of mask provided that it forms a suitable seal around the nose and mouth. The mask (containing the filter) and headstrap are shown on a glass head in profile (left) and face on (center). The right panel shows the filter located within the mask, indicated by the red arrow.

3. Laboratory-based Virus Aerosol Generation and Recovery

We wanted to assess the ability of mask filters to capture exhaled breath aerosols of different sizes in a controlled laboratory system, and then to use this to assess the limit of detection when using masks to capture exhaled virus particles for detection with PCR.

Aerosols were created using a nebulizer which was linked to a particle counter that could detect the numbers of different aerosol particles of different sizes (**Figure 3A**). The distribution of droplet sizes was measured at path lengths of 5 cm and 30 cm, with more smaller particles observed over the longer flow path distance (**Figure 3B**).

Comparing particle detection with and without the presence of a filter allowed us to assess the collection efficiency of the filters, showing >98% efficiency across all particle sizes (**Figure 3C**).



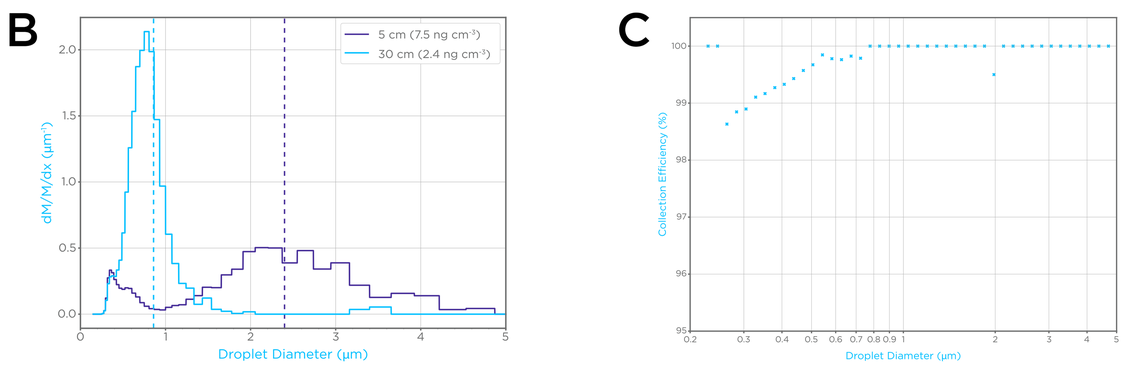
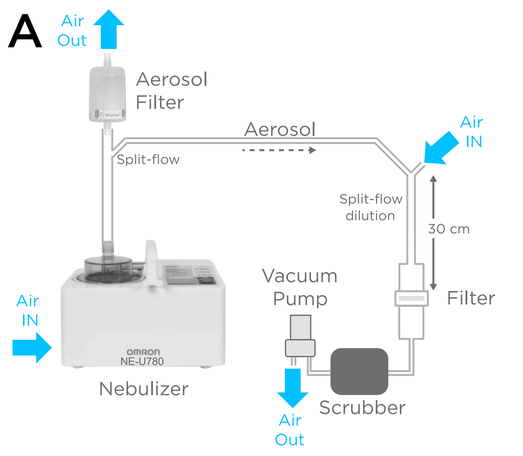


Figure 3: (A) The aerosol generation and detection setup used to assess the efficiency of filters for EBA recovery. (B) Particle mass distribution (probability density by mass; dM/M/dx) of generated EBAs with different path lengths between nebulizer and particle counter. (C) Collection efficiency of the filters assessed by measuring the difference in particle detection with and without a filter present.

A similar setup was used to test the recovery of virus particles from filters. EBAs were generated containing inactivated SARS-CoV-2 or HCoV-NL63 viruses and were captured using a filter over a 30 cm path length (**Figure 4A**).

Using serial dilutions of each virus, we were able to use this setup to generate filters containing estimated numbers of viral particles, which were used with PCR to assess the limit of detection (LoD) for each virus. The resulting LoD for both viruses was approximately 10 copies per filter (**Table 1**). We also used this to assess the half life of inactivated SARS-CoV-2 on filters, which was around 48 hours (**Figure 4B**).

Detection is measured using cycle threshold (Ct), the number of PCR amplification cycles required for detection. A high Ct indicates a lower number of initial copies of virus RNA.



Estimated virus copy number	SARS-CoV-2		HCoV-NL63	
	Mean Ct ± SD	Calculated Copy Number based on Ct	Mean Ct ± SD	Calculated Copy Number based on Ct
1	Undetermined	Undetermined	Undetermined	Undetermined
10	39.9 ± 1.0	16 ± 11	38.45 ± 1.3	14 ± 8
100	37.4 ± 1.53	127 ± 123	35.63 ± 1.3	100 ± 69
1000	33.4 ± 0.81	1437 ± 790	32.41 ± 0.4	801 ± 267

Table 1: Quantitative RT-PCR results of aerosolized SARS-CoV-2 and HCoV-NL63 filter extract samples and calculated virus copies on filters.

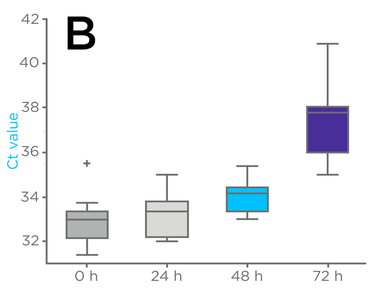


Figure 4: (A) The adapted setup used to test virus-containing aerosol capture on filters and to assess limits of detection. (B) The Ct for SARS-CoV-2 detection rises rapidly for viruses kept on the filter for 48 to 72 hours as virus particles begin the breakdown.

4. Clinical COVID-19 Detection

Having shown that filters can be used to detect coronaviruses to a sufficiently low LoD, we went on to examine the utility of this approach in a clinical setting.

We analyzed EBA samples collected from 47 hospitalized patients with recent positive COVID-19 results, as judged by NPS (**Table 2**). For some patients, NPS samples were collected up to a week before EBA samples. As such, for 39/47 patients we also collected follow-up NPS samples shortly after EBA collection.

Each EBA sample was collected over a period of 30-60 min of regular tidal breathing.

During that time each patient was asked to cough at least 10 times and to speak for at least one minute.

EBA results identified 4/47 cases as COVID-19 positive (sensitivity = 8.5%) (**Figure 5A**).

The follow-up NPS samples showed that 3/39 patients now tested negative using both methods. 2/29 tested positive with both methods (sensitivity = 5.6%) (**Figure 5B**).

These results suggest that, despite the low LoD, EBA collected using filters in masks is not a reliable method of diagnosing COVID-19.

	All Included Patients (47)	True Positives (4)	False Negatives (43)
Age in years (mean ± SD, median)	63 ± 15 (63)	38 ± 16 (38)	65 ± 13 (63)
Female/Male	14/33	2/2	12/31
Shortness of breath (n, days, %)	38, 10.8, 81%	3, 4.0, 75%	35, 11.3, 81%
Cough (n, days, %)	27, 11.5, 57%	1, 7, 25%	26, 11.7, 60%
Fever (n, days, %)	12, 6.9, 26%	3, 3.7, 75%	9, 8.0, 21%
COVID positive CT scan	26/29 (90%)	2/2 (100%)	24/27 (89%)

Table 2: Demographic summary of recruited patients, reported symptoms and additional test results.

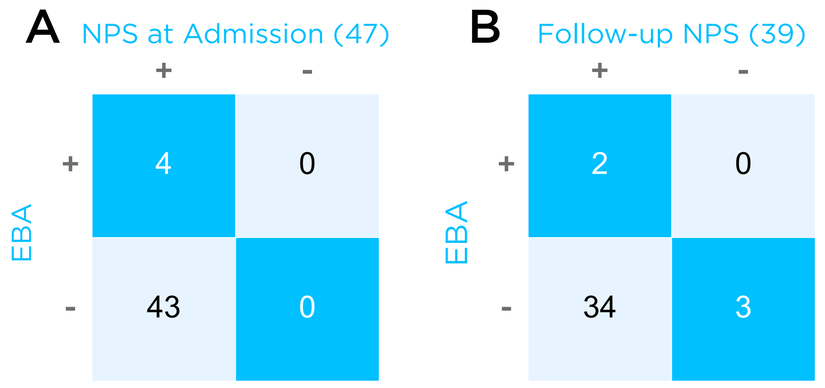


Figure 5: Confusion matrices comparing the results of NPS test for COVID-19 vs. EBA collection. Tests differ only by the method of collection, both use equivalent qRT-PCR approaches for analysis.

(A) Comparison of NPS at admission to EBA collection up to a week later for all 47 patients.

(B) Comparison of EBA to follow-up NPS collected shortly after, for the 39 patients where these data were available.

5. Conclusions

This study demonstrates that EBA collected using electrostatic filters in facemasks is not a suitable alternative to NPS for COVID-19 detection.

However, we have demonstrated that this method has a low LoD for the virus and this may suggest that the lack of detection is indicative of low virus shedding at the relatively late stage of infection when samples were collected.

Other studies have shown that the infectiousness of COVID-19 patients falls rapidly once they develop symptoms. As such, this may indicate that EBA is a useful means of assessing whether patients are still contagious.

The low LoD, if it can be reproduced for other pathogens, may also suggest that EBA could be an effective detection tool for other respiratory infections that have a different infection profile[1,2] (**Figure 6**).

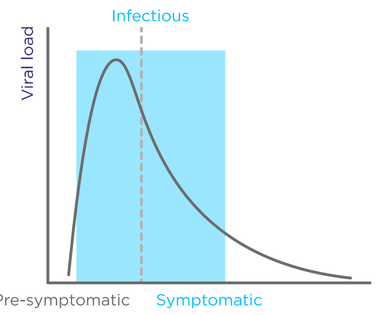


Figure 6: Infectiousness and cycle threshold profiles for COVID-19 infection.

Further Resources

More about Breath Biopsy and COVID-19
owlstonemedical.com/covid-19

Breath Biopsy: The Complete Guide (3rd Edition)
owlstonemedical.com/breath-biopsy-guide

Breath Biopsy Products & Services
owlstonemedical.com/products

This study has recently been published in *Scientific Reports* and can be viewed at owlstonemedical.com/smolinska-2021

6. References

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