

Testing of Worn Face Masks for Timely Diagnosis of SARS-CoV-2 in Hemodialysis Patients

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Testing of worn face masks for timely diagnosis of SARS-CoV-2 in hemodialysis patients

Running title: SARS-CoV-2 on patients' face masks

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Introduction

SARS-CoV-2 is primarily transmitted through aerosolized droplets¹. Patients with COVID-19 exhale SARS-CoV-2 RNA copies at a rate of 10^3 to 10^5 per minute². In the U.S., in-center hemodialysis patients and staff are mandated to wear face masks while staying in the dialysis clinic. Previously, face mask sampling was applied to patients with common cold symptoms³. Recently, a novel method was described to detect exhaled SARS-CoV-2 by using special sampling strips attaching to the inside of a duck-billed mask⁴. We tested the hypothesis that assaying masks worn by in-center hemodialysis patients for SARS-CoV-2 RNA could provide an opportunity to identify infected subjects.

Methods

In this IRB approved exempt study (Western IRB ES-21-003) we collected deidentified masks in a dialysis clinic where hemodialysis patients with COVID-19 are dialyzed in a designated COVID-19 shift. In those patients, SARS-CoV-2 RNA has previously been diagnosed by RT-PCR in nasopharyngeal (NP) swabs. Per our policy, patients stay in the COVID-19 shift until tested negative in two consecutive NP swabs. We also collected masks in patients dialyzed in regular (i.e., non-COVID-19) shifts. Mask wearing is mandatory for everyone, including patients, while staying in the dialysis facility. Three-layer disposable masks were provided to each patient at the time of entry. Upon completion of the session (mostly 3 to 4 hours), patients' masks were collected in individual Ziplock[®] bags and then stored at room temperature for 13 to 24 hours until analysis.

The inner mask surface was swabbed for >30 seconds using a cotton-tipped applicator that was pre-soaked with 100 μ L phosphate buffered saline (PBS). The swab head was then placed in a microcentrifuge tube containing 600 μ L PBS and vortexed for 10 minutes at 1,000 RPM.

RNA extraction and RT-PCR reaction were performed using TaqPath[™] RT-PCR COVID-19 Combo Kit (Thermo Fisher A47814) as described previously⁵. Bacteria MS2 Phage was added to samples prior to RNA extraction. We increased the PCR cycles to 45 for maximum detectability. The multiplex probes anneal to one MS2 sequence and three SARS-CoV-2 target sequences (S gene, N gene, ORF1ab). Test result interpretation is shown in **Figure 1A**.

Results

We collected a total of 138 face masks, 39 from 14 patients with confirmed COVID-19 dialyzed in a designated COVID-19 shift (7 females; median age 59 years [range 32 to 81]), and 99 masks from patients dialyzed in regular shifts. COVID-19 patients provided one to five masks on average 16 ± 10 days (median 15, range 0 to 38) after COVID-19 diagnosis. Eighteen of the 39 masks from COVID-19 patients tested positive for SARS-CoV-2 (**Figure 1B**). Ninety-seven of 99 masks from regular shift patients tested negative (**Figure 1B**); the two positive masks came from an asymptomatic patient who was subsequently diagnosed with COVID-19 by NP swab. Overall, mean cycle thresholds (CT) were around 31.5 for S gene, N gene, and ORF1ab (**Figure 1C**). CT distributions per target are shown in **Figure 1D**.

Discussion

While NP swab RT-PCR is standard for COVID-19 diagnosis, sample collection is uncomfortable to most subjects, neither efficient nor economic for screening purposes, and requires health care professionals.

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3 Our proof-of-principle study shows that face masks worn by in-center hemodialysis patients can harbor
4 SARS-CoV-2. Therefore, we think that assaying of face masks should be explored further, as it could be
5 useful especially when NP swabbing is not feasible or desirable. While the minimum duration of mask
6 use to retrieve SARS-CoV-2 RNA is unknown, it is interesting to note that Ma and colleagues² detected
7 exhaled virus after 5 minutes of breathing into a collection device. Besides RT-PCR testing, viral particles
8 collected from masks can be used for SARS-CoV-2 sequencing.
9

10 There are limitations to our study: First, its exempt nature only allowed us to collect deidentified masks,
11 preventing us from correlating mask results with the time elapsed since onset of COVID-19 symptoms
12 and diagnosis. Second, no NP swabs could be collected concurrently with the masks, precluding
13 quantitation of sensitivity and specificity of mask SARS-CoV-2 testing. However, since the only positive
14 masks in the non-COVID-19 shift came from a patient who was subsequently diagnosed with COVID-19,
15 test specificity is most likely high. Determining test specificity will require concurrent NP swab sampling
16 in COVID-19 patients. The low positive rate in our study may be due to the long lag between COVID-19
17 diagnosis and mask testing, as SARS-CoV-2 shedding declines 4 days after symptom onset⁶.
18
19

20 It is currently unknown if fomite transmission of SARS-CoV-2 via used masks can occur. We are aware of
21 two publications indicating that SARS-CoV-2 virus can remain viable for up to 7 days on surgical masks^{7,8}.
22 Of note, in both reports, mask pieces were soaked in virus transportation medium. We used PBS-soaked
23 cotton-tipped applicators to forcefully swab the masks. These treatments are very different from
24 unintentional, brief touches. Disposable medical masks are usually made of non-woven polypropylene
25 fabric with electrostatic properties that enhances virus retention. Therefore, we think that typical
26 handling of a virus-laden mask is unlikely to result in significant viral transfer.
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29 As incidence and prevalence of COVID-19 is declining, mask testing may lend itself to novel strategies,
30 such as pool testing⁹. Pool testing is particularly efficient in settings with a low disease prevalence¹⁰. This
31 approach will require the development of setting-specific workflows to optimize mask collection and
32 processing.
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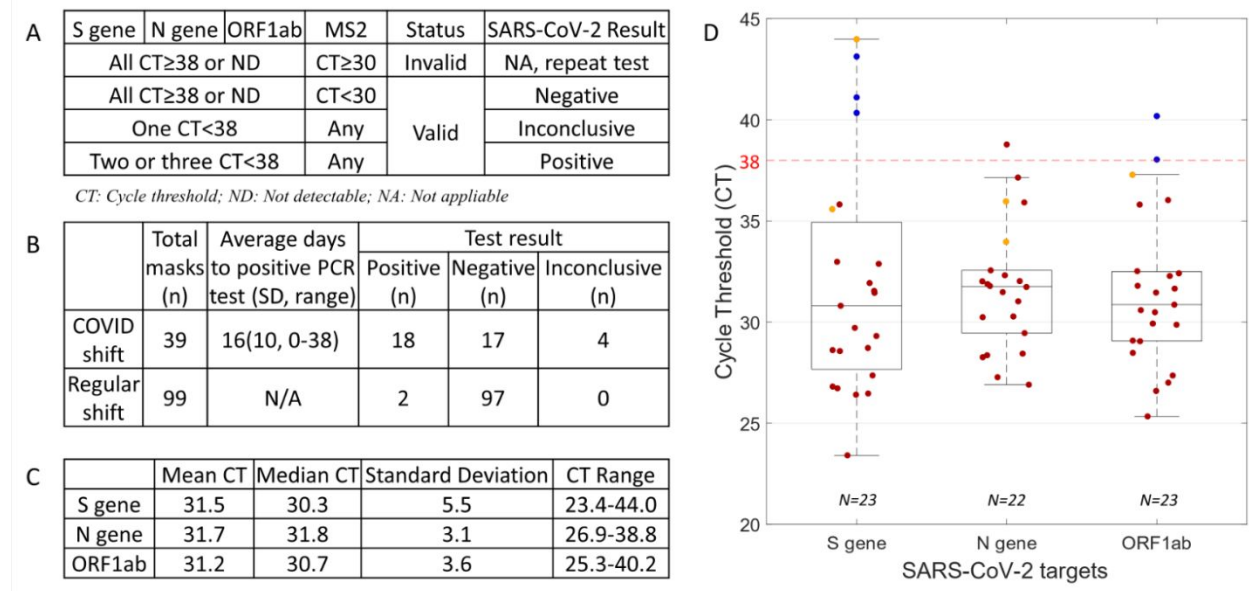
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Figure 1. SARS-CoV-2 testing of face masks worn by in-center hemodialysis patients: Interpretation and results.



Panel A) Interpretation of test results.

Panel B) Test results for a total of 138 face masks collected from patients in COVID-19 and regular shifts.

Panel C) Results for three SARS-CoV-2 RNA targets, S gene, N gene and ORF1ab, isolated from face masks worn by patients who tested positive for SARS-CoV-2 by nasopharyngeal swab. Note that two positive samples from regular shift are included in the calculation.

Panel D) Cycle threshold (CT) distribution for three SARS-CoV-2 targets for all 138 masks (undetectable CTs are excluded). Positive (red), negative (blue), and inconclusive (orange) results per definition shown in panel A. The red dashed line indicates the CT cut-off for SARS-CoV-2 PCR validation.