Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

American Journal of Infection Control 50 (2022) 890–897

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Detection of SARS-CoV-2 in exhaled air using non-invasive embedded strips in masks

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Key words:
SARS-CoV-2
COVID-19
Personal protective equipment
Face masks
qRT-PCR analysis

A B S T R A C T

Background: SARS-CoV-2 emerged in 2019 and resulted in a pandemic causing millions of infections worldwide. Gold-standard for SARS-CoV-2 detection uses quantitative RT-qPCR on respiratory secretions to detect viral RNA (vRNA). Acquiring these samples is invasive, can be painful for those with xerostomia and other health conditions, and sample quality can vary greatly. Frequently only symptomatic individuals are tested even though asymptomatic individuals can have comparable viral loads and efficiently transmit virus.

Methods: We utilized a non-invasive approach to detect SARS-CoV-2 in individuals, using polyvinyl alcohol (PVA) strips embedded in KN95 masks. PVA strips were tested for SARS-CoV-2 vRNA via qRT-PCR and infectious virus.

Results: We show efficient recovery of vRNA and infectious virus from virus-spiked PVA with detection limits comparable to nasal swab samples. In infected individuals, we detect both human and SARS-CoV-2 RNA on PVA strips, however, these levels are not correlated with length of time mask was worn, number of times coughed or sneezed, or level of virus in nasal swab samples. We successfully cultured and deep-sequenced PVA-associated virus.

Conclusions: These results demonstrate the feasibility of using PVA-embedded masks as a non-invasive platform for detecting SARS-CoV-2 in exhaled air in COVID-positive individuals regardless of symptom status.

SARS-CoV-2 emerged in 2019 and has since spread worldwide, resulting in millions of infections and deaths. Early in the pandemic, diagnostic testing was extremely limited, and only available for those with symptoms as it was predicted that one had to be symptomatic to be infected.[1] We have since learned that a large portion of infections are entirely asymptomatic, and that asymptomatic and presymptomatic individuals can harbor high levels of virus and contribute to transmission and spread.[2,3] Testing capacity has since increased, including surveillance testing of high-risk and congregant communities (eg, skilled nursing facilities, college campuses, prisons, etc.) to detect asymptomatic/presymptomatic infections and prevent spread and outbreaks.[4]

SARS-CoV-2 diagnostic tests rely on detecting viral RNA or antigen from oral (oropharyngeal swab, buccal swab, saliva, sputum etc.) or nasal samples (nasopharyngeal, mid-turbinate, etc.).[5] Collection of these samples can be invasive and are often from a single time point. Additionally, while detection of viral RNA within the nose/mouth of an individual demonstrates that they are currently infected, it is unclear whether all RNA positive individuals are capable of infecting others.[6] Here we propose testing of face masks, allowing us to determine the amount of exhaled

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Funding/support: The authors acknowledge that funds for the project were provided by a grant from the German Federal Ministry of Health (BMG), facilitated via the World Health Organization. External partners include Dr. Brian Montague (University of Colorado), Dr. Kellie Hawkins (Denver Health Medical Center), and Jeannine Reiss and Joni Triantis Van Sickle (Colorado State University). Open access of this article is sponsored by the World Health Organization.
Conflicts of interest: None to report.

https://doi.org/10.1016/j.ajic.2022.01.010
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SARS-CoV-2 as a more accurate measure of transmission potential across individuals. Testing of face masks is non-invasive, and most individuals are already wearing masks for many hours a day, making them a convenient sampling platform.

In our proof-of-concept study, we use KN95 masks embedded with polyvinyl alcohol (PVA) strips. PVA is commonly used in cosmetic products, has a good safety profile, and is approved as hypo-allergenic and nontoxic for humans.[7] PVA-embedded masks theoretically capture large and small particles exhaled from individuals while breathing talking, coughing, sneezing, etc. all of which are hypothesized to contain infectious virus and drive transmission.[6,8,9] Colleagues successfully developed PVA-based face mask testing for respiratory *Mycobacterium* and has recently adapted it for COVID-19 testing in hospitalized patients.[10,11] Our study builds on this work by sampling participants through the course of their infection, allowing us to compare the dynamics of nasal swab and face mask positivity over time. Additionally, we developed techniques to recover infectious virus from face mask samples, and deep-sequence SARS-CoV-2 virus from face masks at sufficient depth and coverage to identify rare variants.

METHODS

Study sites and participant enrollment

PCR-confirmed COVID-19 cases (regardless of symptom status) were enrolled at multiple sites throughout Colorado (noted below) and followed for either 2 days or over 5 to 14 days, depending on test positivity. We asked the participants to wear the masks provided by the study team every day during follow-up for pre-specified time periods (either 2 days, or 5-14 days depending on the study design), and design 2 participants additionally provided one self-administered nasal swab per day.

Design I PCR-positive study participants from Occupational Health at the University of Colorado, Anschutz Medical Campus and Denver Health Medical Center were enrolled between late December 2020 and early April 2021 (24 participants total), and design 2 participants were enrolled at Colorado State University between April and July 2021 (16 participants total). Study inclusion criteria were individuals ≥ 18 years of age of any sex, that had tested PCR-positive for SARS-CoV-2 via their institution's normal screening procedures. Exclusion criteria were less than 18 years of age, unable to wear a face mask

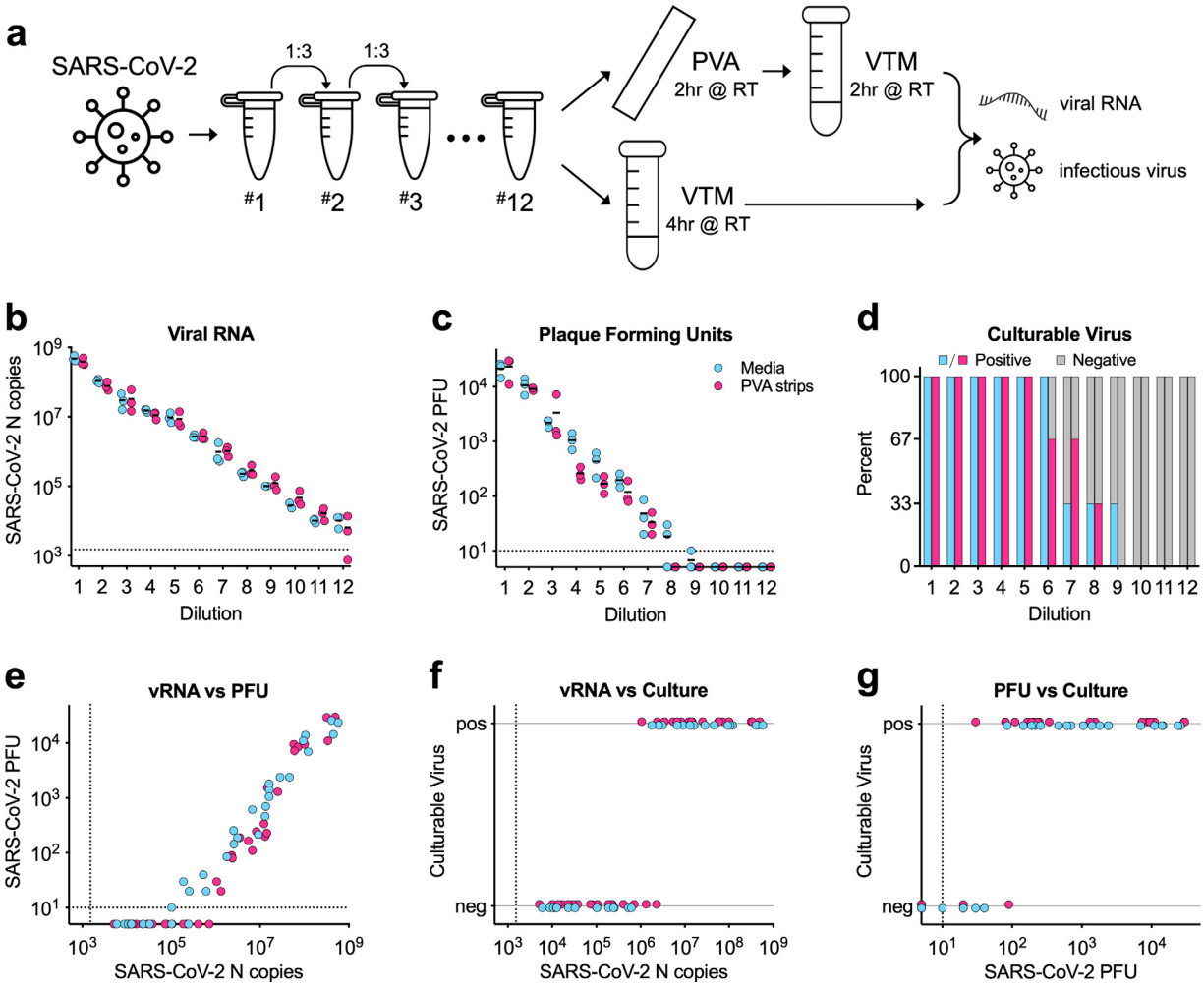

Fig 1. SARS-CoV-2 is efficiently recovered from polyvinyl alcohol (PVA) strips. (A) SARS-CoV-2 was diluted and spiked onto PVA strips or into viral transport media. Samples were incubated at room temperature for 2 hours, PVA strips were transferred into viral transport media, then incubated an additional 2 hours at room temperature. Viral RNA (vRNA) and infectious virus were measured. (B) vRNA (N1) was measured via qPCR, infectious virus measured by (C) plaque assays and (D) culturing virus. (E-G) relationships between vRNA and infectious virus. Virus spiked into media (blue), virus spiked onto PVA strips (pink). Experiment was performed in biological triplicate. Dashed lines represent limits of detection

due to underlying condition, not able to consent, or pregnancy. Additionally, individuals with any signs of severe disease present at time of enrollment (eg, difficulty breathing, pain when breathing, tightness of chest, etc.) were excluded from participation. Participants provided informed consent to participate in the study. The participants were told that this is a research study and that the mask strips are a novel experimental sampling method but were not otherwise sensitized prior to enrollment. This study was reviewed and approved by the Colorado Multiple Institutional Review Board (COMIRB) protocol numbers 20-2084 and 21-2823 and by the World Health Organization Ethics Review Board under protocol number CERC.0096.

Spike-on experiments

PVA strips (1 × 9 cm) were produced by 3D printing and provided by Dr. Michael Barer.[11,12] In a biosafety level 3 laboratory, SARS-CoV-2 virus (2019-nCoV/USA-WA1/2020 strain) was diluted in either infection media (DMEM +1% FBS) or phosphate buffered saline (PBS) and pipetted either directly onto PVA strips or into tubes containing viral transport media (Hanks Balanced Salt Solution, 1% FBS, 50 mg/mL gentamicin, 250 ug/mL amphotericin B/fungizone). PVA strips were dried inside the biosafety cabinet for the indicated length of time depending on experiment (Fig 1 = 2 hour drying time, Supplementary Fig 4 = 1, 2, 6, 12, 24, and 48- hour drying times) then transferred to 15ml conical tubes containing VTM. Tubes (both those containing PVA strips, and those containing spiked on virus) were incubated at room temperature the indicated length of time, then processed for viral RNA and infectious virus as described below.

Participant sample collection and processing

Two PVA strips were affixed to the inside of a KN95 mask using small squares of double-sided tape; an "upper" strip (level with nostrils), and a "lower" strip (level with mouth). Depending on the participant's face size and shape, the actual positioning and proximity of the strips relative to the nostrils and mouth might vary across participants. All participants wore masks for each of the assigned lengths of time (30 minutes, 1 hour, 2 hours, 3 hours), and stored the masks in a sealed bag until processed by lab personnel (ranging from 1-30

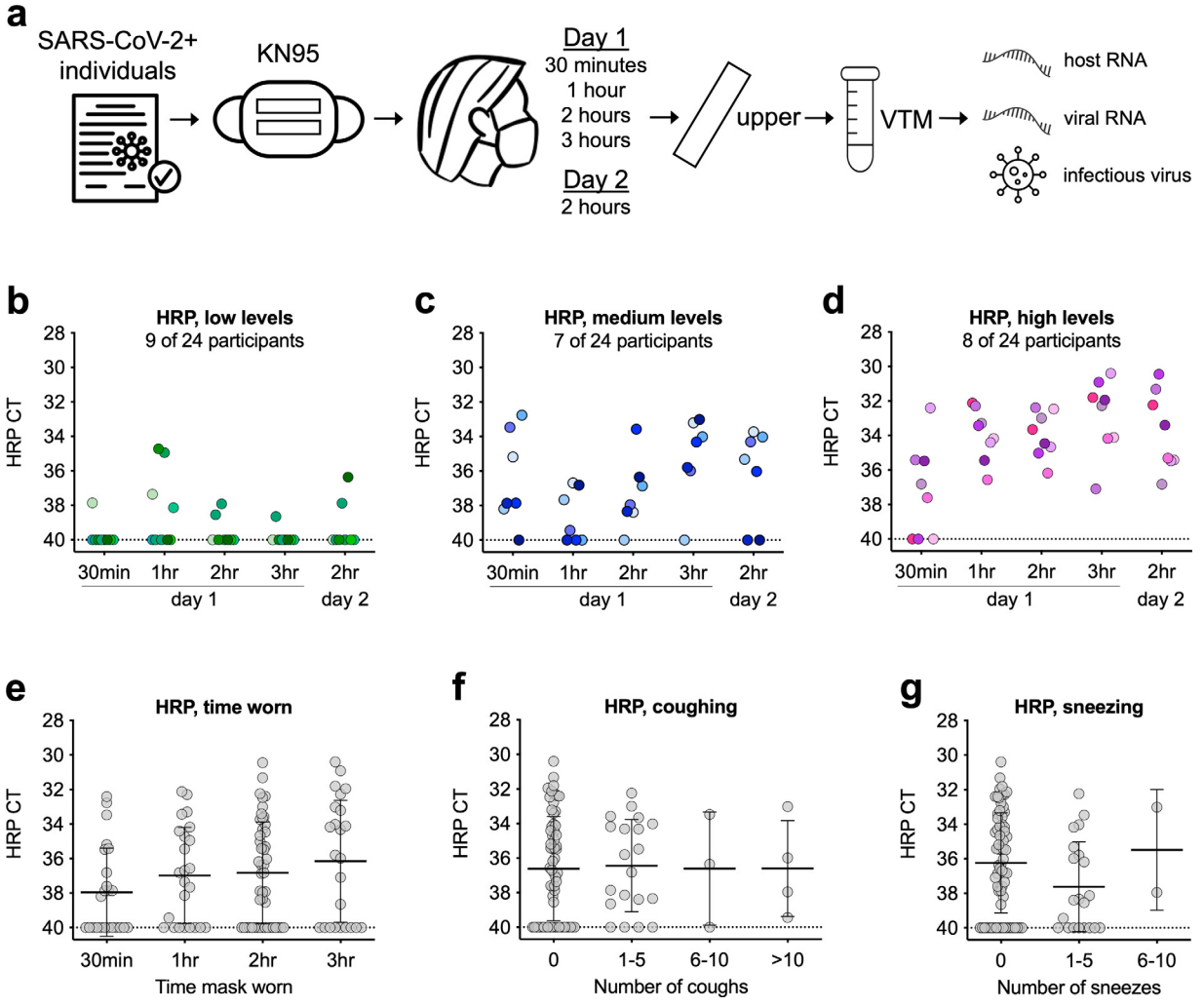

Fig 2. PVA-associated host material is highly variable across participants and activities. (A) SARS-CoV-2 infected individuals (n = 24) were enrolled into our study, where they were provided with 5 KN95 masks embedded with PVA strips to be worn over two days. After wearing, strips were harvested and tested for host RNA, viral RNA, and infectious virus. (B-D) Participants were divided into 3 groups based on the amount of human RNase P (HRP) detected on the upper PVA strip: (B) low (2 ≤ samples positive for HRP), (C) medium (3-5 samples positive for HRP, CT < 32.5), (D) high (4-5 samples positive for HRP). Colors in B-D represent different participants. (E) Samples were stratified based on length of time worn. Individuals completed a survey after wearing each mask reporting types and frequencies of many common behaviors. Samples were stratified based on the (F) number of coughs and (G) number of sneezes that occurred while wearing the mask. Dashed lines represent limits of detection.

hours). Participants self-administered nasal swabs by inserting swab ~0.5 in into 1 nostril, gently pressing it around the inside of the nostril for ~10sec, then placing it into a tube containing 2 mL VTM. PVA strips were harvested from KN95 masks using sterile scissors and forceps to cut the strips from the double-sided tape and placing each strip into a tube containing VTM. Tubes containing PVA strips in VTM were incubated at room temperature for at least 30 minutes to allow the PVA to dissolve. Samples were stored at 4°C prior to RNA extraction.

RNA extraction and qRT-PCR

Tubes containing virus-spiked VTM, PVA strips and nasal swabs were vortexed, then RNA extracted from 200μl supernatant using the Omega Mag-Bind Viral DNA/RNA 96 Kit on a KingFisher Flex magnetic particle processor (ThermoFisher Scientific). qrt-PCR was performed using the EXPRESS One-Step SuperScript qrt-PCR reagents and N1, N2, E, and human RNAse P primer/probes as previously described.[13]

Plaque assay and virus culturing

Plaque assays were performed on African green monkey kidney (Vero) cells (ATCC CCL-81), as previously described.[13] Serially diluted samples were inoculated onto cells for one hour, overlaid with tragacanth medium, incubated for 2 days, then fixed and stained with 30% ethanol and 0.1% crystal violet. Virus was cultured by adding sample to confluent Vero cells for one hour, adding growth media, and incubating cells for 3-4 days or until cytopathic effect was visible in positive control wells.

Symptom and activity reporting

Study data were collected and managed using Research Electronic Data Capture capture tool hosted at Colorado Clinical and Translational Sciences Institute at University of Colorado Anschutz Medical Campus.[14,15] Research Electronic Data Capture is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for data integration and interoperability with external sources. Subjects completed an electronic enrollment questionnaire in which they reported their demographic information, pre-existing conditions, and illness history. Subjects then completed daily electronic questionnaires regarding their symptoms (if any), activities and comfort while wearing the mask with embedded strips, and overall opinions regarding masks. Questionnaires included questions regarding warning signs for severe disease that triggered an auto-alert to study investigators. If alerted, we immediately contacted the participant and a) asked the participant to stop wearing the masks, and b) advised the participant to seek medical care if warning signs were confirmed.

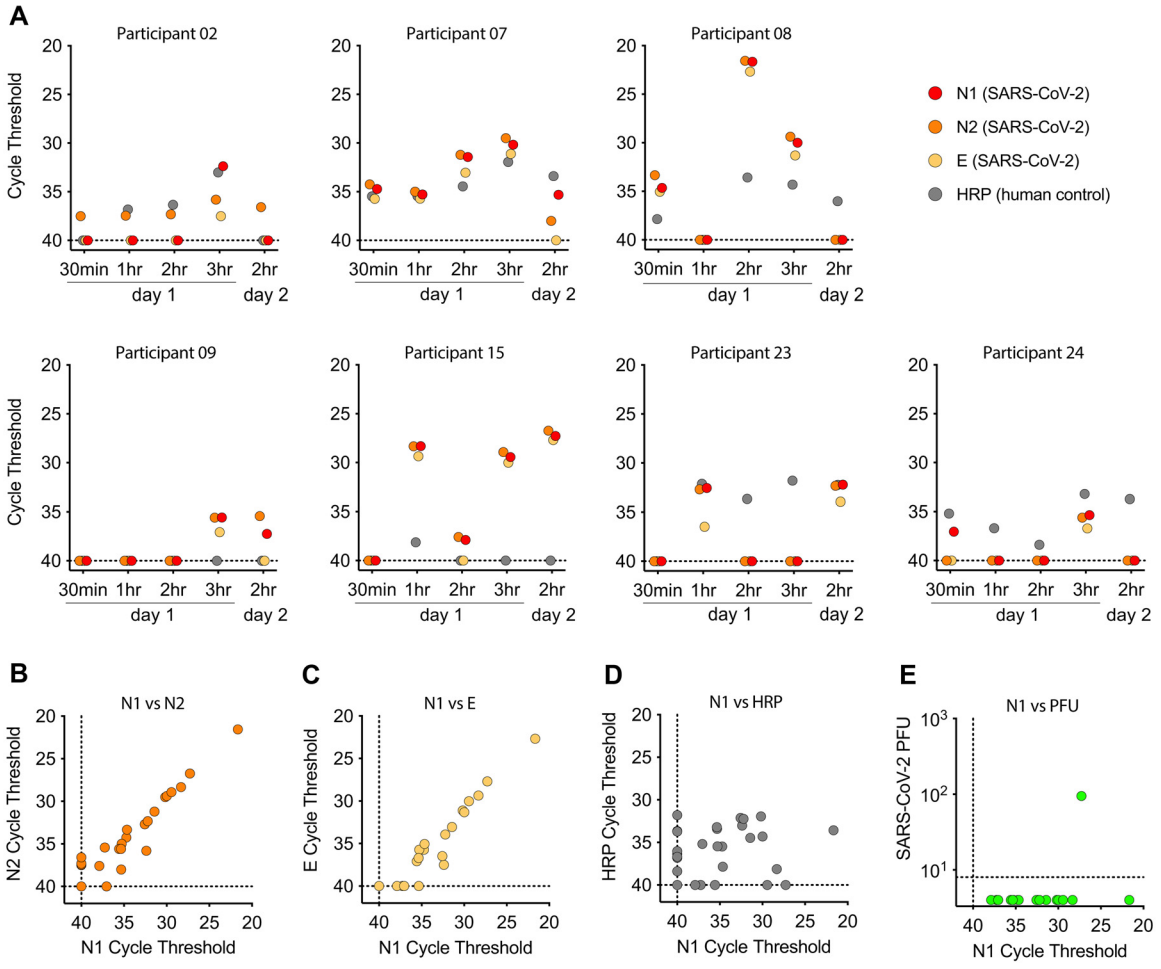

Fig 3. Detection of SARS-CoV-2 on PVA strips. (A) RNA was extracted from upper PVA strips of masks worn by vRNA-positive participants (n = 7), and qRT-PCR was performed using SARS-CoV-2 N1, N2, E, and HRP primer-probes. Relationship between N1 cycle threshold and B) N2 cycle threshold, (C) E cycle threshold, (D) HRP cycle threshold from all time points from the 7 positive participants. (E) Relationship between N1 cycle threshold and SARS-CoV-2 plaque forming units of the N1 positive samples. Dashed lines represent limits of detection.

Next-generation sequencing and analysis

Sequencing and analyses were performed as previously described.[13] Briefly, cDNA was generated, PCR amplification was performed using ARTIC primers and Q5 High-Fidelity polymerase, products were purified and libraries were prepared using KAPA HyperPrep kit and unique index primers. Libraries were sequenced on the Illumina MiSeq V2 using 2 × 250 paired-end reads. Sequencing data were processed, and quality checked.

RESULTS

Infectious SARS-CoV-2 can efficiently be recovered from PVA strips

Before testing clinical participant samples, we first wanted to determine our ability to recover SARS-CoV-2 virus from PVA strips using conditions similar to what would be required for participant samples (Fig 1). SARS-CoV-2 virus was 3-fold serially diluted in DMEM containing 1% FBS, then added directly to PVA strips or tubes containing viral transport media. PVA strips were fully dried and incubated at room temperature for 2 hours to replicate the time of participants wearing masks (this doesn't however take into account time sample transport time). Dried PVA strips were then transferred to tubes containing VTM and incubated for 2 hours at room temperature (~22 °C), mimicking the time required to transport samples to the laboratory. We demonstrated highly efficient recovery of viral RNA from PVA strips as compared to virus-spiked media, even at low levels of virus (Fig 1B). Despite PVA strips drying for 2 hours, we efficiently recovered infectious virus, as measured by both plaque assays and culturing (Fig 1C & D). We observed similar relationships between PVA-associated and virus-spiked media, when comparing viral RNA and plaque forming units (Fig 1E), viral RNA and culturable virus (Fig 1F) and plaque forming units and culturable virus (Fig 1G), indicating that the PVA strips, nor the processing impacts any of our measures of quantifying virus.

Upper PVA strips from participant masks contains more host material

Each mask worn by study participants contained 2 PVA strips, an upper strip at nostril level, and a lower strip at mouth level. We first

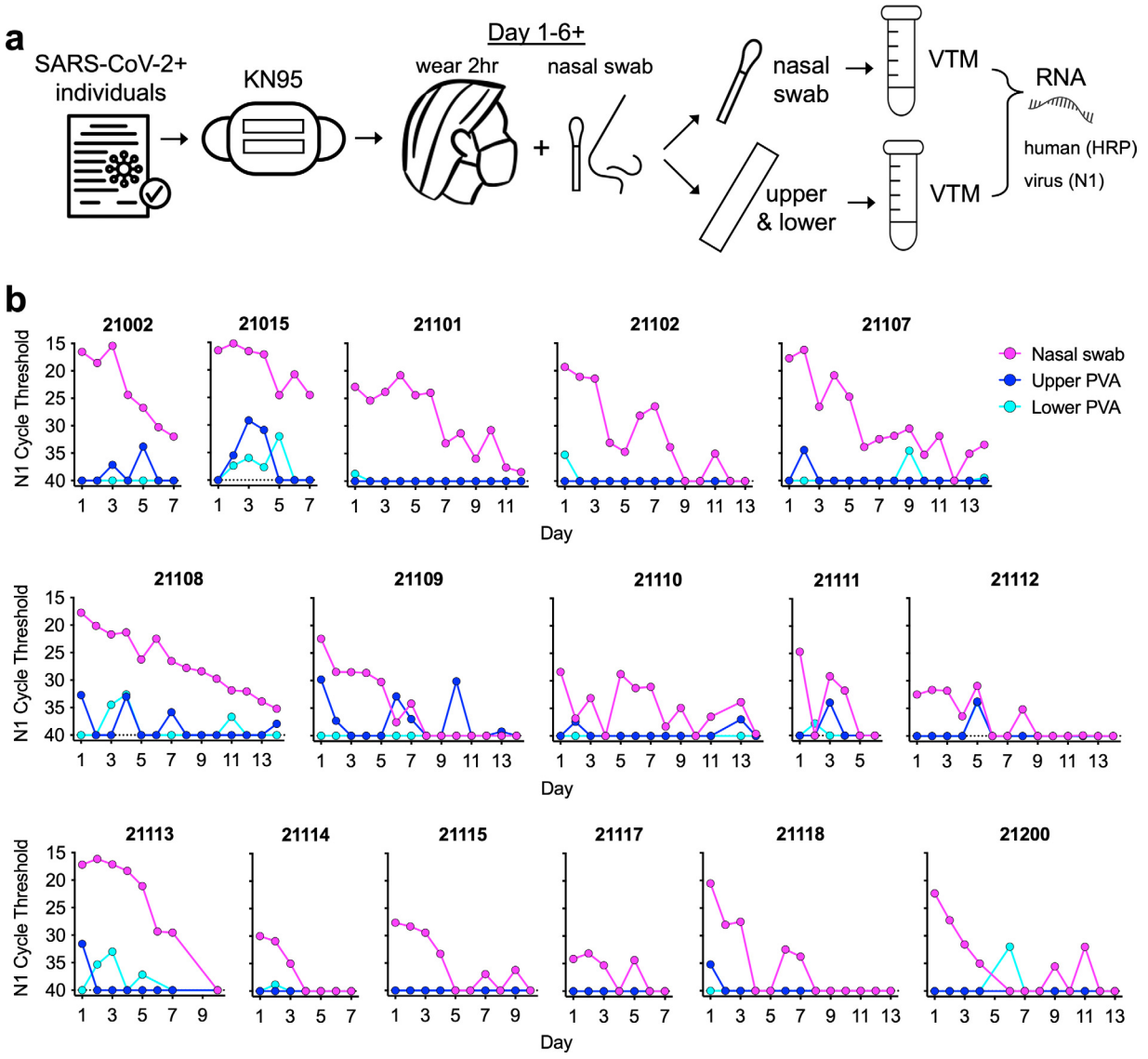

Fig 4. SARS-CoV-2 on PVA strips and nasal swabs from infected participants. (A) SARS-CoV-2 positive participants enrolled in the study (n = 16) where they were provided KN95 masks embedded with 2 PVA strips which was worn for 2 hours each day, in addition to a self-sampled nasal swab. Masks and nasal swabs were processed and tested for human RNase P (HRP) and viral RNA (N1). (B) Nasal swab (pink), upper PVA strip (blue) and lower PVA strip (cyan) N1 cycle threshold values. Dashed line represents limit of detection.

wanted to determine if either of the strip positions capture more host material, and therefore might be more likely to contain SARS-CoV-2 virus. To test this, participants wore KN95 masks containing 2 PVA strips for 2 hours (design 1), then both PVA strips were harvested, RNA extracted, and tested for host (human RNase P) and bacterial (16S) RNA via qRT-PCR (Supplementary Fig 1A). In all individuals tested, the upper strip contained more human RNA relative to the lower strip (Supplementary Fig 1B & C). In most individuals (8 out of 9), the upper strip also contained more bacterial 16S RNA compared to the lower PVA strip (Supplementary Fig 1D & E). Because the upper strips contained significantly ($P < .05$) more human and bacterial RNA, we therefore chose to only test the upper strip from participant samples. Interestingly, the amount of host and bacterial RNA found on either strip location did not correlate with number of times coughed, sneeze, or talking during mask wearing (Supplementary Fig 1F-K).

Time worn, coughing and sneezing do not correlate with amount of host material on PVA strips

Individuals that recently tested positive for SARS-CoV-2 were enrolled into our proof-of-concept study (design 1). They were provided 5 masks containing 2 PVA strips. On day one post-enrollment, participants wore a mask for 30 minutes, 1 hour, 2 hours and 3 hours, and on day 2, they wore a single mask for 2 hours (Fig 2a). On day 2, all PVA strips were harvested into VTM and tested for host RNA (HRP) and viral RNA, and infectious virus. Participants completed a survey after each mask wearing episode (5 surveys total), noting any activities and behaviors that occurred during the time they were wearing the mask (coughing, sneezing, talking, exercising, etc.). The amount of host RNA on upper PVA strips varied greatly across individuals, with 38% (9/24) having no to very little detectable HRP (Fig 2b), 29% (7/24) having more frequently detectable and medium levels of HRP (Fig 2c) and 33% (8/24) having consistently high levels of HRP (Fig 2d). No significant trends ($P > .05$) were observed in HRP levels across all individuals and all days and lengths of time masks were worn, when comparing length of time mask was worn (Fig 2E), number of times the participant coughed (Fig 2F) or sneezed (Fig 2G).

Recovery of SARS-CoV-2 from PVA strips on participant masks

Of the 24 study participants (design 1), 17 (71%) did not have detectable SARS-CoV-2 vRNA on any of their mask PVA strips, however most had detectable HRP on their upper PVA strip from at least one time point, which did not correlate with activity (coughing and or sneezing) (Supplementary Fig 2). The remaining 7 participants (29%) tested positive for SARS-CoV-2 on at least one of their samples, however the frequency, and level of virus varied highly across individuals, length of time worn, and day (Fig 3A). The level of viral RNA on the PVA strips did not correlate with length of time worn, number of coughs, or sneezes, or whether the wearer talked while wearing the mask (Supplementary Fig 3). We observed strong correlations between viral N1 and N2 (Fig 3B), and N1 and E results (Fig 3C), with N1 being the most sensitive. We found no correlation between PVA HRP and N1 levels (Fig 3D). Samples that were positive for SARS-CoV-2 N1 vRNA were tested for infectivity using a plaque assay (Fig 3E). Despite some samples containing high levels of viral RNA (N1 cycle threshold < 28), only one sample (participant 15, day 2) contained infectious virus (Fig 3E). We suspect that because day 1 masks were not processed until day 2, the virus lost infectivity by drying for 24+ hours. We tested this hypothesis by diluting a biologically relevant amount of virus in PBS (which would be more like human respiratory secretions than our standard dilution media which has buffering capacity), added it to PVA strips, allowed it to dry at room temperature up to 48 hours, then tested for vRNA and infectious virus (Supplementary Fig 4A). We found no impact of drying time on recovery of viral RNA (Supplementary Fig 4B) even after 48 hours of incubation at room temperature (which is much longer the time experienced with participant samples), however virus quickly lost infectivity on PVA strips after only 2 hours at room temperature (Supplementary Fig 4C). Therefore, to improve the likelihood of recovering infectious virus for subsequent experiments, we sought to minimize the time between wearing of masks, and processing PVA strips into VTM.

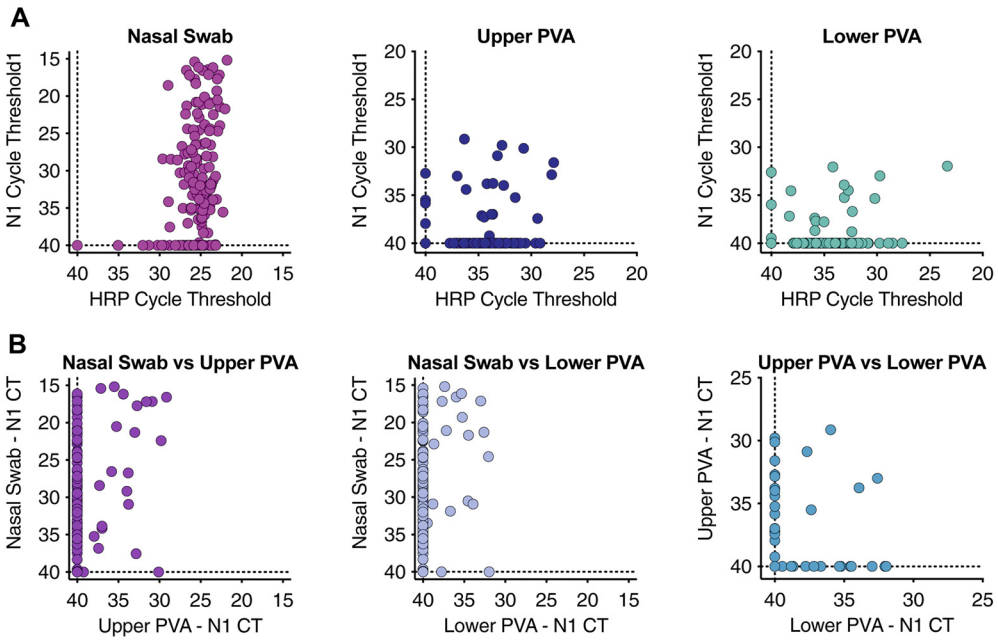

Fig 5. Relationship between SARS-CoV-2 on nasal swab and PVA strips. (A) Relationship between human RNase P (HRP) and viral RNA (N1) on nasal swab, upper PVA and lower PVA strips. (B) Relationship of viral RNA (N1) between nasal swab and upper PVA, nasal swab and lower PVA, and upper PVA and lower PVA. Dashed line represents limit of detection.

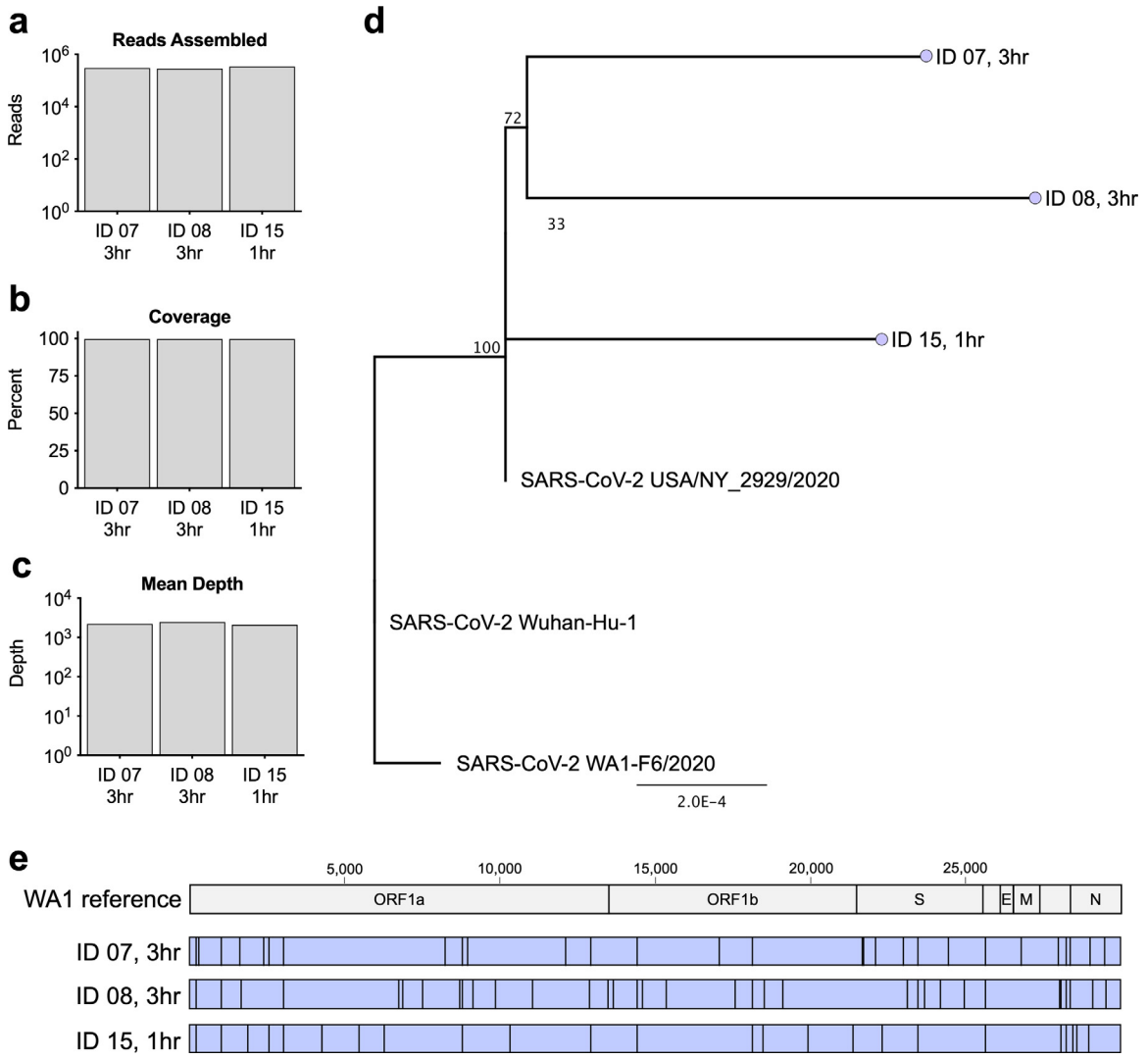

Fig 6. Full genome sequencing of PVA-associated SARS-CoV-2. Whole genome sequencing was performed on RNA isolated from PVA strips from 3 participants (IDs 07, 08, and 15), and were analyzed based on (A) reads assembled, (B) genome coverage and (C) mean depth. (D) PhyML tree constructed using Tamura-Nei distance model, including both transitions and transversions, in Geneious Prime. Numbers at nodes indicate bootstrap confidence based on 1,000 replicates. Distance matrix was computed, and the tree was visualized in Geneious Prime. (E) Consensus nucleotide sequence of each participant sample was compared to the WA1 SARS-CoV-2 reference genome, and single nucleotide polymorphisms relative to WA1 are shown as black lines.

SARS-CoV-2 on nasal swabs and PVA strips over time

`From our proof-of-concept design 1 study (Fig 3), we did not know if the PVA-negative samples were because the participant was no longer infected. Therefore, we designed a larger, more comprehensive study (design 2), where a new cohort of SARS-CoV-2 infected individuals were enrolled (n = 16), wore a PVA-embedded mask for 2 hours daily, and performed a self-collected nasal swab daily (Fig 4A). In addition to the daily nasal swab, we tested both upper and lower PVA samples to identify trends between nasal and oral exhaled virus. We observed variable patterns of SARS-CoV-2 N1 vRNA on nasal swabs and PVA strips over time and across individuals (Fig 4B). When all participants and time points were aggregated, we see nasal swabs have the highest rate of host and viral RNA positivity, and upper and lower PVA strips have similar positivity rates (Supplementary Fig 5A & B). Day-wise nasal swab and PVA human RNA positivity is steady over time (Supplementary Fig 5C & E), whereas viral RNA detection decreases (Supplementary Fig 5D & F). Participants fell into 4 groups based on positivity: nasal swab positive only (eg, 21115, 12.5% (2/16)), nasal swab and upper PVA positive (eg, 21002, 25% (4/16)), nasal swab and lower PVA positive (eg, 21200, 25% (4/16)) and nasal swab and upper and lower PVA positive (eg, 21015, 37.5% (6/16)). Interestingly, we saw some time-points where there was no detectable viral RNA on the nasal swab, but detectable virus on the upper or lower PVA strip (participant 21,109 day 10, and 21200 day 6, respectively) (Fig 4B). The PVA-associated virus in these samples likely came from the mouth/saliva, or virus that is deeper in the nasal passage than the swab reaches.

We saw no relationship between the level of human (HRP) and viral (N1) RNA on nasal swabs, upper PVA strips, and lower PVA strips (Fig 5A). We hypothesized that the amount of virus on PVA strips would be correlated to the level of virus within the nasal swab, however that was not supported by our data, nor was the relationship between virus on the upper and lower PVA strips (Fig 5B), demonstrating that something other than level of nasal virus determines the amount of exhaled virus.

Deep-sequencing of PVA-associated SARS-CoV-2

We selected three SARS-CoV-2 vRNA-positive PVA samples from three individuals (design 1) to perform whole genome deep sequencing (Fig 6). Each sample had over 10^5 reads (Fig 6A), resulting in >99% genome coverage (Fig 6B), with a mean depth of over 10^3 reads/nucleotide (Fig 6C), which is sufficient coverage and depth to identify rare variants. Phylogenetic analysis revealed the three participant samples are more closely related to one another than three reference genomes (Fig 6D). The participant sequences contain many shared and unique single nucleotide polymorphisms relative to the WA1 SARS-CoV-2 reference genome (Fig 6E).

DISCUSSION

SARS-CoV-2 rapidly spread throughout the world, resulting in over 200 million infections despite aggressive control efforts, highlighting the virus's ability to be efficiently transmitted between people. Despite this, little is known regarding transmission, infectiousness, contagiousness, and why some individuals are super spreaders and others are not. While host factors are thought to contribute to super spreader events, the emergence of more transmissible variants reveal that virus biology can also impact transmission potential.[16,17]

SARS-CoV-2 diagnostic testing uses oral and/or nasal samples, and modeling has revealed that higher nasal/oral viral load increases transmission potential.[18] However, the factors that predict whether an infected individual will exhale virus or be contagious to others are still poorly understood. In our proof-of-concept study, we utilized non-invasive mask-based testing of SARS-CoV-2 infected individuals to determine the amount of virus in their nasal passage, and the amount being produced onto the inside of their mask. Importantly, we found no relationship between the amounts of virus in these 2 samples, consistent with results from similar studies.[19] This suggests there are likely multiple factors impacting transmission potential (both host and viral), including vaccination and booster status, prior SARS-CoV-2 infection, virus sequence, and days post-exposure or symptom onset.[11,20] Importantly, our ability to culture infectious virus from PVA strip samples allows us to characterize (growth, pathogenesis, neutralization, etc.) the virus potentially being transmitted.

Our study is currently being expanded to follow index cases and their contacts in multiple high risk congregant settings (college campus residence halls, middle school classrooms, etc.), to better understand the relationship between the level of exhaled virus (as measured on PVA strips embedded in masks), and transmission to others. Additionally, using deep sequencing on nasal and PVA mask samples, we hope to identify variants resulting in more efficiently exhaled and transmissible viruses. Mask wearing is a public health and preventive measure that will likely be part of our lives for the foreseeable future, so PVA-based mask testing has the potential to be an ideal noninvasive platform to capture respiratory pathogens for diagnosis and surveillance. This system can be quickly implemented with the emergence of new platforms and pathogens.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2022.01.010.

References

1. Mercer TR, Salit M. Testing at scale during the COVID-19 pandemic. *Nat Rev Genet*. 2021;22:415–426.
2. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis. *PLoS Med*. 2020;17: e1003346.
3. Jefferson T, Spencer EA, Brassey J, et al. Transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) from pre and asymptomatic infected individuals. A systematic review. *Clin Microbiol Infect*. 2021;28:178–189.
4. Jatt LP, Winnett A, Graber CJ, et al. Widespread severe acute respiratory coronavirus virus 2 (SARS-CoV-2) laboratory surveillance program to minimize asymptomatic transmission in high-risk inpatient and congregate living settings. *Infect Control Hosp Epidemiol*. 2020;41:1331–1334.
5. Kevadiya BD, Machhi J, Herskovitz J, et al. Diagnostics for SARS-CoV-2 infections. *Nat Mater*. 2021;20:593–605.
6. Wang CC, Prather KA, Sznitman J, et al. Airborne transmission of respiratory viruses. *Science*. 2021:373.
7. Review, CI, *Safety assessment of polyvinyl alcohol as used in cosmetics: final report*. 2014. Accessed June 28, 2022. https://www.cir-safety.org/sites/default/files/PVA.pdf.
8. Stadnytskyi V, Anfinrud P, Bax A. Breathing, speaking, coughing or sneezing: what drives transmission of SARS-CoV-2? *J Intern Med*. 2021;290:1010–1027.
9. Stadnytskyi V, Bax CE, Bax A, Anfinrud P, et al. The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. *Proc Natl Acad Sci U S A*. 2020;117:11875–11877.
10. Williams CM, Abdulwhhab M, Birring SS, et al. Exhaled Mycobacterium tuberculosis output and detection of subclinical disease by face-mask sampling: prospective observational studies. *Lancet Infect Dis*. 2020;20:607–617.
11. Williams CM, Pan D, Decker J, et al. Exhaled SARS-CoV-2 quantified by face-mask sampling in hospitalised patients with COVID-19. *J Infect*. 2021;82:253–259.
12. Al-Taie A, Han X, Williams CM, et al. 3-D printed polyvinyl alcohol matrix for detection of airborne pathogens in respiratory bacterial infections. *Microbiol Res*. 2020;241: 126587.
13. Gallichotte EN, Quicke KM, Sexton NR, et al. Early adoption of longitudinal surveillance for SARS-CoV-2 among staff in long-term care facilities: prevalence, virologic and sequence analysis. *Microbiol Spectr*. 2021 e0100321.
14. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform*. 2019;95: 103208.
15. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377–381.
16. Lakdawala SS, Menachery VD. Catch me if you can: superspreading of COVID-19. *Trends Microbiol*. 2021;29:919–929.
17. Plante JA, Mitchell BM, Plante KS, et al. The variant gambit: COVID-19′s next move. *Cell Host Microbe*. 2021;29:508–515.
18. Goyal A, Reeves DB, Cardozo-Ojeda EF, Schiffer JT, Mayer BT. Viral load and contact heterogeneity predict SARS-CoV-2 transmission and super-spreading events. *Elife*. 2021;10:e63537.
19. Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med*. 2020;26:676–680.
20. Sriraman K, Shaikh A, Parikh S, et al. Non-invasive adapted N-95 mask sampling captures variation in viral particles expelled by COVID-19 patients: implications in understanding SARS-CoV2 transmission. *PLoS One*. 2021;16: e0249525.