SARS-CoV-2 and other respiratory pathogens are detected in continuous air samples from congregate settings.

Mitchell D. Ramuta[1], Christina M. Newman[1], Savannah F. Brakefield[1], Miranda R. Stauss[2], Roger W. Wiseman[1,2], Amanda Kita-Yarbro[3], Eli J. O'Connor[4], Neeti Dahal[5], Ailam Lim[5], Keith P. Poulsen[5], Nasia Safdar[6], John A. Marx[6], Molly A. Accola[6], William M. Rehrauer[1,6], Julia A. Zimmer[7], Manjeet Khubbar[7], Lucas J. Beversdorf[7], Emma C. Boehm[9], David Castañeda[9], Clayton Rushford[8], Devon A. Gregory[8], Joseph D. Yao[11], Sanjib Bhattacharyya[7], Marc C. Johnson[8], Matthew T. Aliota[9], Thomas C. Friedrich[10], David H. O'Connor[1,2 ‡], Shelby L. O'Connor[1,2‡*]

[1] Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, USA

[2] Wisconsin National Primate Research Center, Madison, WI USA

[3] Public Health Madison & Dane County, Madison, WI, USA

[4] EAGLE School, Fitchburg, WI USA

[5] Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA

[6] University of Wisconsin Hospitals and Clinics, Madison, WI, USA

[7] City of Milwaukee Health Department Laboratory, Milwaukee, WI

[8] Department of Molecular Microbiology and Immunology, University of Missouri, School of Medicine, Columbia, MO, USA

[9] Department of Veterinary and Biomedical Sciences, University of Minnesota, Twin Cities, Minneapolis, MN, USA

[10] Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA

[11] Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

‡These authors contributed equally.

*Correspondence can be addressed to:

Shelby O'Connor

slfeinberg@wisc.edu

555 Science Drive

Madison, WI USA 53711

Abstract

Two years after the emergence of SARS-CoV-2, there is still a need for better ways to assess the risk of transmission in congregate spaces. We deployed active air samplers to monitor the presence of SARS-CoV-2 in real-world settings across communities in the Upper Midwestern states of Wisconsin and Minnesota. Over 29 weeks, we collected 527 air samples from 15 congregate settings and detected 106 SARS-CoV-2 positive samples, demonstrating SARS-CoV-2 can be detected in air collected from daily and weekly sampling intervals. We expanded the utility of air surveillance to test for 40 other respiratory pathogens. Surveillance data revealed differences in timing and location of SARS-CoV-2 and influenza A virus detection in the community. In addition, we obtained SARS-CoV-2 genome sequences from air samples to identify variant lineages. Collectively, this shows air surveillance is a scalable, cost-effective, and high throughput alternative to individual testing for detecting respiratory pathogens in congregate settings.

Introduction

Viral testing and surveillance have been a challenge throughout the COVID-19 pandemic in the United States. To date, nasal swab-based testing has predominated. Such testing did not reliably and consistently detect SARS-CoV-2 upon its arrival in the United States. First-generation PCR assays were problematic and slow to deploy[1]. A massive increase in the need for PCR testing strained supply chains and laboratory capacity, leading to lengthy turnaround times[2,3]. The development of saliva-based SARS-CoV-2 testing in 2020 reduced the impact of certain supply chain bottlenecks, but laboratory capacity and test availability remained a problem in the United States[4]. Lower-cost, point-of-care antigen tests became available in late 2020 but were not widely used for at-home testing until the arrival of the Omicron Variant of Concern in late 2021. The explosive spread of Omicron was unprecedented; on January 10, 2022, the United States reported the highest single-day COVID-19 case number of over 1.3 million new cases and had a seven-day case average three times greater than the previous highest peak recorded in January 2021[5,6]. Once again, testing laboratories could not scale to meet surging demand and at-home antigen test results, which are rarely reported to public health authorities, were not considered in case counts. As a result, existing testing systems for COVID-19 have provided case counts that are, at best, crude estimates for disease burden and transmission risk. Highlighting this discordance, serological data estimates that for every diagnosed case of COVID-19, there are 4.8 undiagnosed SARS-CoV-2 infections[7].

Such swab-based estimates of community infection rates are likely to become less accurate in the future. Antigen testing will cause fewer people to seek out formal diagnostic testing from providers who report test data to public health authorities; indeed, the United States federal government recently began the distribution of 500 million antigen tests without requiring mandatory results reporting[8]. There has been a growing concern that the mental and physical exhaustion caused by COVID-19, often referred to as “pandemic fatigue,” could reduce willingness to seek out testing when symptomatic, particularly if a positive test result is linked to undesirable outcomes such as mandatory isolation[9].

Accurate estimates of SARS-CoV-2 risk are especially important in congregate settings where individuals with varying degrees of risk are in close contact. Highlighting the importance of these settings, the United States invested more than $12 billion in March 2021 to expand testing in schools, workplaces, long-term care facilities, and underserved congregate settings[10]. Evidence of increased SARS-CoV-2 risk from testing programs can be used as an impetus to intensify mitigation measures,

such as recommending or requiring facial masking or increased testing. Conversely, such measures can be relaxed when the risk of infection is low. This has led some to advocate for frequent, routine testing of everyone in congregate settings[11–14]. Many different approaches to high throughput testing have been deployed in support of such comprehensive testing[4,15–17], but these are expensive and difficult to scale and maintain. There is also a risk that such resource-intensive testing programs will perpetuate inequalities in the distribution of COVID-19 testing access[18–23].

Alternative "environmental" testing strategies that do not rely on individualized testing could provide a more accurate, rapid, and efficient assessment of SARS-CoV-2 infection risk in congregate settings. To date, wastewater-based surveillance for SARS-CoV-2 has received the most attention as an environmental testing strategy[24–29]. Viral RNA is shed in the feces of 30-66% of individuals with COVID-19, regardless of their symptoms[30–32], allowing SARS-CoV-2 to be detected in wastewater samples. Untreated wastewater collected at municipal wastewater treatment plants includes fecal and liquid waste from households in a sewershed and represents an efficient pooled sample that can provide information on asymptomatic and symptomatic SARS-CoV-2 infections.

Wastewater-based surveillance can provide population-wide surveillance data for large geographic regions but mainly relies on fixed sampling locations, limiting its ability to provide spatial resolution within a sewershed. Collecting sewage from individual buildings is possible, and sample collection can be challenging due to differences in the design and complexity of wastewater infrastructure. Furthermore, wastewater surveillance that relies on sample collection from wastewater treatment plants does not capture communities with decentralized systems (e.g., septic tanks) or sites where sewage is pre-treated for decontamination before reaching the wastewater treatment plant (e.g., hospitals). This is a limitation because the prevalence of COVID-19 and risk of transmission may vary substantially across different congregate settings in a community. There is still a need to develop agile and mobile surveillance technologies to collect hyperlocal data with higher resolution than is possible with wastewater.

Air surveillance is an alternative form of environmental sampling for SARS-CoV-2. Passive and active air sampling techniques have been used for air surveillance of viruses, bacteria, and fungi that are released in respiratory droplets and aerosols when infected individuals talk, cough, sneeze, and breathe[33–36]. Notably, the United States Department of Homeland Security established the BioWatch

Program in 2003 to use active air samplers as routine environmental monitoring systems to detect specific biological threats to combat bioterrorism[37].

Continuous air sampling has key advantages over widespread individual testing and wastewater testing for surveillance in congregate settings. Air surveillance is cost-effective compared to frequent swab-based testing of individuals. Air samples contain a mixture of exhaled components from many individuals and can capture pathogen-containing droplets and aerosols from infectious individuals, enabling virus detection independent of symptoms, test-seeking behavior, and access to swab-based testing. In contrast to wastewater surveillance, active air samplers can be easily moved to different locations, making it possible to collect surveillance data with ultrahigh spatial resolution (e.g., a single room in a building).

Several studies have shown the utility of active air samplers to detect aerosols containing SARS-CoV-2[38–43] in controlled settings and locations with known SARS-CoV-2 cases. Horve et al. (2021) demonstrated consistent detection of heat-inactivated SARS-CoV-2 virus at an aerosol concentration of 0.089 genome copies per liter of air (gc/L) when air samples were collected in a room-scale experiment during an eight-hour interval[38]. Another study compared the effectiveness of surface and bioaerosol sampling methods to detect SARS-CoV-2 and showed active air samples detected SARS-CoV-2 in 53% of the samples when run for 1-2 hours in hospital rooms of COVID-19 patients, while passive air sampling and surface swabs detected SARS-CoV-2 in only 12% and 14% of samples, respectively[39]. Parhizkar et al. (2021) used active air samplers to assess the relationship between COVID-19 patient viral load and environmental viral load in a controlled chamber over three days. Increases in patient viral load were associated with lower cycle threshold (Ct) values detected in near (1.2 meters) and far (3.5 meters) air samplers[40]. Lastly, a study demonstrated the utility of using active air samplers to track the presence and concentration of virus in air longitudinally during COVID-19 isolation periods in student dormitories. The study observed a significant increase in Ct values for COVID-19 positive students after their first test, as well as in environmental samples as individuals recovered indicating a reduction in virus presence[41]. These studies provide proof of concept on the feasibility of using active air samplers to detect SARS-CoV-2. However, each study was performed in a controlled environment occupied by COVID-19 positive individuals.

Here, we evaluate whether active air samplers can be used for prospective air surveillance of SARS-CoV-2 in real-world congregate settings, where pathogen-containing aerosols are likely present at a

much lower concentration, and the presence of positive individuals is unknown. This study addresses a key knowledge gap for how active air samplers perform as routine pathogen monitoring systems in real-world settings. We demonstrate that it is feasible to use active air samplers for air respiratory pathogen detection and sequencing across different types of congregate settings.

Results

Study design

From July 19, 2021, to February 9, 2022, continuous air samplers were deployed in several public locations to survey SARS-CoV-2 in the environment of real-world settings. We used Thermo Fisher Scientific AerosolSense Samplers for daily and weekly air surveillance at places considered to be high-risk for close-contact, indoor SARS-CoV-2 transmission. Air cartridges were collected and tested for SARS-CoV-2 RNA by quantitative reverse transcription PCR (RT-qPCR) and transcription-mediated amplification (TMA) (Figure 1). Several different RT-qPCR and TMA assays were used to test samples for viral RNA (vRNA) throughout the study, depending on the location of the test site and the availability of testing at the time. Further details on the cut-off values used for calling air samples positive, inconclusive, or negative are described in the methods section. We developed a user-friendly workflow to collect air cartridge metadata, upload test data, and report results to surveillance sites within 24-48 hours of receiving air cartridges for testing (Figure 1), enabling non-technical staff (e.g., custodial staff, students) to exchange and catalog cartridges easily and accurately.

SARS-CoV-2 detection in community settings

To demonstrate the utility of air surveillance in real-world settings, we chose a diversity of community locations for placement: a campus coffee shop, hospital, office, campus athletic training facility, brewery taproom, cafeteria, bar, two preschools, four K-12 schools, and two shelters located throughout Wisconsin and Minnesota. We collected a total of 527 air cartridges from the 15 locations to test for the presence of SARS-CoV-2 genomic material (Table 1). Four hundred sixty-six (88.4%) air samples were collected from testing sites in Dane County, Wisconsin, 26 (4.9%) from Minneapolis, Minnesota, 19 (3.6%) from Rochester, Minnesota, and 16 (3.1%) from Milwaukee, Wisconsin. During the 29 weeks, Dane County experienced a moderate-to-high transmission rate of COVID-19 cases

despite having a high county-wide vaccination rate (>65% adults with two doses) (Supplementary Figure 1). Increases in COVID-19 cases were observed following the emergence of Delta and Omicron Variants of Concern in the community. An emergency mask mandate was instituted by Public Health Madison Dane County on August 19th, 2021 and extended throughout the entire study. The order required every individual aged two and older to wear a face-covering in most public enclosed spaces, including K-12 schools. Exceptions were made in spaces where all people were known to be vaccinated.

Throughout the study, we detected a total of 106 SARS-CoV-2 positive and 52 inconclusive air cartridges (an inconclusive result is defined when at least one of the PCR targets is positive while at least one of the PCR targets is negative). We were able to intermittently identify SARS-CoV-2 positive air cartridges at 14 of the 15 surveillance sites, even when intensive risk mitigation strategies were implemented by public health (Table 1). Together, this provides evidence that SARS-CoV-2 can be detected in continuous air samples collected from a variety of prospective, real-world settings.

By testing air samplers in these real-world settings, it was impossible to know the SARS-CoV-2 status of every person who spent time in the proximity of the samplers, but we were able to retrospectively correlate air surveillance data with reported test results during a prolonged COVID-19 outbreak at one of the testing sites (Figure 2). The first confirmed case was reported by an individual who quarantined at home after experiencing symptoms on day 10, had a diagnostic test specimen collected two days later, and received test results on day 14. Individuals who congregated in the same room were quarantined after the case was reported and no one from the room tested positive during that time. Three colleagues, considered to be close contacts with the first COVID-19 positive person but worked in different rooms, tested positive with antigen tests while in the building on days 14, 15, and 16. Two of the individuals were symptomatic and one was asymptomatic at the time of testing. Two more rooms were quarantined and seven people tested positive while at home. A fifth individaul tested positive on-site on day 23 after being in the buidling for the entire prior week; close contacts in their room were quarantined and eight individals tested positive while in quarantine. Air samples were positive before the first confirmed case of COVID-19 and throughout the outbreak that resulted in a total of 20 confirmed cases; SARS-CoV-2 genetic material was detected in an air sample for the first time between days 5 and 7, preceding the first confirmed case by 7 days. Air samples collected after day 23 were either inconclusive or negative for SARS-CoV-2. No reported cases were observed in the

building during this time. No air sample was collected from day 28-30 because the air cartridge was inserted improperly, leading to a machine error. This suggests that congregate risk during this outbreak could have been estimated with air sampling data alone had individual testing not been available or widespread, and that air sampling provided an early indication of SARS-CoV-2 transmission risk. It should be noted that, at the time of this outbreak, we did not have sufficient data on air sampling accuracy to make recommendations to the affected congregate setting to intensify risk mitigation.

Extending the duration of continuous air sampling

Daily air surveillance programs are resource-intensive and expensive. To reduce the cost and complexity of surveillance programs, we tested the feasibility of extending the sampling interval. Over the course of five weeks, two adjacent AerosolSense instruments were deployed to either run continuously (~168 hours) or daily (~24 hours) over several days. Air samples were gathered throughout the week, nucleic acids were isolated simultaneously, and tested with two RT-qPCR Center for Disease Control and Prevention (CDC) assays targeting SARS-CoV-2 N1 and N2. During the first week of sampling, both air samplers detected SARS-CoV-2 genetic material, showing continuous air sampling for a week captured similar SARS-CoV-2 genetic material, determined by Ct values, when compared to more resource-intensive daily sampling (Figure 3). Weekly and daily air sample results were concordant for the next two weeks; air samples were either negative or inconclusive. Continuous air samples were negative for the last two weeks, while daily sampling identified one inconclusive sample during each of these weeks. However, each of the inconclusive samples had high N2 Ct values of 39.1 and 39.4, respectively. This suggests that congregate settings can use either daily or weekly sampling to achieve a balance between cost and turnaround time while maintaining detection sensitivity.

Expanding detection to additional respiratory pathogens

To explore whether pathogens other than SARS-CoV-2 are detected in the same collected air sample, we tested them for the presence of nucleic acids of 40 other viral, bacterial, and fungal respiratory pathogens using the TrueMark Respiratory Panel 2.0 Array Card on a QuantStudio 7 Pro Instrument[44]. We assessed the limit-of-detection (LOD) for each TrueMark assay using contrived air samples to

determine pathogen-specific thresholds for calling samples positive or negative. Contrived air samples were created and tested in quadruplicate by spiking the TrueMark Respiratory Panel 2.0 Amplification Control (Thermo Fisher Scientific) plasmid into pooled air samples at concentrations of 0, 1.25, 12.5, 50, and 250 copies per reaction. Air samples were collected for 48 hours from an empty office to minimize any background pathogens present in the samples (see methods section for more details). Pathogen-specific thresholds were determined by averaging the Cycle relative threshold (Crt) values of positive replicates at the lowest dilution concentration with at least 75% positive replicates. Cycle relative threshold values listed in Supplementary Table 1 were used as cut-off values for positivity for the detection of each pathogen.

From October 25, 2021, to February 9, 2022, air samples were collected weekly from eight sites in Dane County, Wisconsin: a campus coffee shop, a preschool, an office space, a campus athletic training facility, and four K-12 schools. Semi-quantitative RT-PCR was performed on 104 air samples using this TrueMark Respiratory 2.0 Panel. During the 15 weeks of air surveillance, we detected 16 different respiratory pathogens across the eight sites (Supplementary Table 2). Commensal or transiently commensal respiratory tract microbes were frequently detected in air samples at each site, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Moraxella catarrhalis* (Figure 4). The panel also detected respiratory pathogens associated with illness in school-aged children, including adenovirus, human coronavirus OC43, Epstein-Barr virus, cytomegalovirus, influenza A virus, parainfluenza virus 3, respiratory syncytial virus A, and SARS-CoV-2. Certain pathogens, such as human bocavirus, were consistently detected only in settings where there were young children, consistent with its widespread distribution in this population, highlighting that different types of congregate settings have distinctive air surveillance pathogen signatures (Figure 4B)[45].

The pattern of influenza A virus (IAV) nucleic acid detection was especially striking. The Wisconsin Department of Health Services issued a health alert on November 30, 2021, noting increased IAV activity among college and university students[46]. As shown in Figure 5, we detected IAV nucleic acids in the air collected from two AerosolSense samplers deployed on the University of Wisconsin-Madison campus beginning the week of November 10, 2021. Air collected from both of these samplers contained IAV nucleic acids from mid-November 2021 to January 2022. One sampler, located in a campus coffee shop, was negative for IAV beginning the week of December 22, 2021, through the week ending January 12, 2022 (Figure 5A); this coincided with the end of the UW-Madison fall

academic semester and holiday break. During this time, the coffee shop was closed for customers from December 17, 2021, to January 18, 2022, but the building was still open for repairs and cleaning. We continued to detect IAV nucleic acids collected by the sampler in the training facility, as student-athletes and staff remained on campus for training and competition during the holiday break. Strikingly, IAV nucleic acids were only sporadically detected in air samplers located on non-campus community sites in Dane County, Wisconsin. In contrast, SARS-CoV-2 nucleic acids were frequently detected at testing sites across the community (Figure 5B). Dane County experienced a high transmission rate of COVID-19 during this time and the detection of SARS-CoV-2 positive air samples increased at testing sites following the emergence of Omicron. Overall the differential detection of IAV and SARS-CoV-2 nucleic acids is consistent with the localized known IAV campus outbreak and wide-spread SARS-CoV-2 transmission.

Sequencing of SARS-CoV-2 RNA from collected air samples

Throughout the pandemic, deep sequencing of SARS-CoV-2 RNA extracted from clinical samples and wastewater has played a crucial role in monitoring viral evolution and tracking variants of concern. We used two sequencing strategies to obtain partial and near-full genome SARS-CoV-2 sequences from 11 air samples to demonstrate the feasibility of genotyping SARS-CoV-2 from collected air. Sequencing efforts were focused on air samples with low Ct values from congregate settings where individuals often removed their masks to eat and drink (e.g. taproom and shelters).

Targeted sequencing was performed on nine samples collected from two AerosolSense samplers in a brewery taproom in Minnesota between November 22, 2021, and January 25, 2022, using primers targeting the SARS-CoV-2 spike gene receptor-binding domain (RBD)[26]. Consensus sequences from four samples collected between November 22 and December 13, 2021, all contained the characteristic S:L452R and S:T478K variants associated with the Delta lineage that predominated at this time (Table 2). Interestingly, one cartridge collected genetic material that had two rare consensus variants, S:F456L and synonymous S:F562F, suggesting that sequencing can detect unexpected variants in air samples and could be used for detecting newly emerging variants of concern in congregate settings. Six air samples collected between December 30, 2021, and January 25, 2022, all contained characteristic S:K417N, S:N440K, S:G446S, S:S477N, S:T478K, S:E484A, S:Q493R, S:G496S, S:Q498R, S:N501Y, S:Y505H, S:T547K variants associated with the Omicron BA.1 lineage coinciding

with the emergence of Omicron in the region (Table 2). In early January, we detected both Delta and Omicron sequences in one of the air samples. These data support that virus genetic material collected by air samples parallels the longitudinal detection of the same lineages transmitted in the community.

The remaining two additional samples with Ct values below 32 collected from two shelters in Milwaukee, Wisconsin, from December 21, 2021, to January 7, 2022, were examined using the ARTIC protocol followed by Illumina sequencing. Sequence coverage was incomplete for both samples, with 28% and 4% of the sequences having low coverage or missing data. However, there was enough information to assign both samples to the Omicron BA.1 lineage (Table 2).

Discussion

An increasing number of public health organizations have employed environmental surveillance methods, in conjunction with individual clinical testing, to provide data on SARS-CoV-2 prevalence, monitor viral evolution, and track viral variants in communities[24,29,47,48]. Environmental surveillance tools could help public health make data-driven decisions for implementing COVID-19 interventions and allocating resources within a community. This study demonstrates the feasibility of using active air samplers for environmental pathogen surveillance in real-world settings, even while intensive risk-mitigation strategies are implemented. Air surveillance may improve our ability to identify pathogen-containing aerosols present in congregate settings to assess the risk of transmission with high spatial resolution, providing a more complete picture to public health officials.

An especially attractive element to air surveillance is its relative cost-effectiveness compared to intensive, individual testing in congregate settings. For example, Wisconsin received more than $175 million in United States federal funding to support SARS-CoV-2 testing of individual teachers, staff, and students in 2021[49]. There are 5,987 public and private elementary and secondary schools in Wisconsin, plus 2,716 preschool and child development centers[50,51]. These are geographically distributed throughout the state, such that air sampling results in schools could be generally representative of the communities in which they are located. At approximately $5,000 per air sampler (https://www.thermofisher.com/order/catalog/product/AEROSOLSENSE), each school in the state could be equipped with an air sampler for approximately $43.5 million. Assuming daily testing for 180 school days per academic year at a cost of $115 per sample ($40 per cartridge and $75 per

test), a testing program would be cost-similar to the investment in individual testing ($180 million)[52]. A more strategic design that tests daily during the high-risk period for respiratory pathogens (ie., mid-November through February) and weekly during the remainder of the school year would be considerably less expensive. Moreover, the possibility of substituting low-cost nylon flocking material for the AerosolSense Capture Material, as described by others, could enable cartridge recycling, substantially reducing both cost and plastic waste[38]. While there would undoubtedly be other unforeseen costs of deploying air sampling at scale (e.g., creation of high volume data management solutions), the ability to task-shift air sampling cartridge management to staff with no scientific training makes air sampling more accessible and scalable than individual testing with specialized personnel. Furthermore, air sampling programs could help lessen the burden on individuals participating in testing programs compared to current swab-based surveillance testing.

Networks of air samplers deployed as described here could also play a role in improving public health resilience to new and emerging respiratory diseases. Had a nationwide network existed in the United States prior to the arrival of SARS-CoV-2, the spread of the virus through space and time could have been more accurately evaluated. Moreover, adding continuous air sampling in the future at ports of entry and, potentially, aboard international aircraft could intercept vessels and passengers harboring worrisome respiratory pathogens. Improvements in technologies that enable real-time, highly multiplexed pathogen detection and genotyping could be leveraged with air sampling to improve quarantine effectiveness. Consider the arrival of the Omicron variant: it was initially reported to the World Health Organization by South African authorities on November 24, 2021[53]. The first confirmed Omicron case, however, occurred weeks earlier in a sample collected on November 9, 2021. Additionally, continuous genomic surveillance of air by targeted spike or whole-genome sequencing from international travelers arriving in the United States could have shortened the window of first detection of Omicron in this country. Establishing a network of air surveillance programs now could provide an early warning for the arrival of future SARS-CoV-2 variants, as well as future novel respiratory viruses of concern.

We used RT-qPCR analysis for detecting low levels of pathogen nucleic acids captured in air samples. Quantitative RT-PCR assays of nucleic acids in a test tube provide semi-quantitative information on the viral load present in ambient air, but these viral load data should be interpreted with care. The exact environmental viral load and duration of exposure are unknown because of the large sampled air

volumes collected during daily and weekly sampling intervals in this study. Furthermore, the amount of genetic material collected by active air samplers depends on many factors, including the location of the sampler, ventilation capacity, the amount of virus shed by each infected individual, the number of infected individuals in an area, and the dimensions of the indoor environment. We are now optimizing the placement of air samplers by evaluating carbon dioxide levels, air exchange, and movement data with low-cost sensor arrays to define locations most likely to sample air from a large number of individuals in a congregate setting.

Additionally, RT-qPCR analysis cannot distinguish whether an air cartridge is collecting infectious virus or genetic material that does not pose a risk for infection. We did not attempt to culture SARS-CoV-2 or any other pathogens from air cartridges in this study to determine if infectious virus can be isolated from the nucleic acid positive samples. Several studies have attempted to culture SARS-CoV-2 and other viruses from air samples with mixed results[42,43,54,55]. Furthermore, previous studies have shown that the air sampling method and capture media can affect viral integrity[56]. AerosolSense samplers use an accelerating slit impactor to collect aerosol particles on dry filter capture substrate. Air sampling methods that rely on impactors and filters are not optimal for maintaining virus viability because of damage caused by impaction forces and dehydration during the collection process. Live virus recovery from continuous air samples would be valuable, as it might potentiate culture and expansion of unknown pathogens with greater sensitivity.

After two years of COVID restrictions, there is pushback against public health measures to counteract virus transmission[9,57]. One component of this resistance is that guidelines issued at the national, state, or even municipal level do not necessarily reflect hyperlocal risk within specific congregate settings: an individual school, sports arena, bar, etc. Air sampling provides a measure of risk with this level of granularity. However, care must be taken when interpreting and sharing air sampling data. In some settings, stakeholders may choose to be liberal in disclosing air sampling results, sharing this information with employees, customers, visitors, and others so they can individually modulate their risk mitigation. In other settings, public health and testing laboratories may work directly with the leadership of congregate settings to couple air sampling data with action. For example, one of the county public health departments involved in this work offers enhanced testing to sites where high levels of SARS-CoV-2 is detected in the air, while a second public health department created a flow

chart describing how schools might respond to positive air sampling data if there are no known cases of SARS-CoV-2 in a given school (Supplementary Figure 2).

Additionally, the brewery taproom implemented a vaccine mandate on December 10, 2021, after observing an increase in SARS-CoV-2 detection in air samples and COVID-19 cases in the community. The vaccine mandate required customers to show proof that they received all recommended doses in their primary series of COVID-19 vaccines or had a negative COVID-19 test within the last 72 hours for indoor dining. No air samples collected between December 13, 2021, and December 30th, 2021, were positive for SARS-CoV-2. However, SARS-CoV-2 was detected in 72% of the air samples collected following the emergence of Omicron. These data reiterate that vaccinated individuals infected with SARS-CoV-2 can shed virus[58].

We did not have sequencing data available from the brewery taproom in real-time. In retrospect, sequencing and RT-qPCR data could have been used to help make data-driven decisions to adapt the risk mitigation strategy. Adjustments to the COVID-19 policy could have included increasing the ventilation or expanding the vaccine mandate to require booster doses that have been shown to improve protection against Omicron[41,59]. However, even with these data available, congregate settings may be hesitant to increase risk mitigation strategies past the most stringent guidelines set out by the CDC and local public health. Furthermore, some settings may have no appetite for COVID-19 risk mitigation regardless of air surveillance results. Environmental surveillance in these settings may nonetheless be valuable to public health alone, allowing them to anticipate and respond quickly to surges in respiratory disease[60]. In fact, in settings where diagnostic testing for SARS-CoV-2 is limited by pandemic fatigue and apathy towards risk minimization measures, air sampling could be exceptionally useful in providing baseline data on respiratory virus levels that would otherwise be impossible to obtain.

Taken together, these results show that continuous air surveillance with active air samplers can unambiguously detect respiratory pathogens, including SARS-CoV-2, in congregate settings. Similar to the National Wastewater Surveillance System recently established by the US Centers for Disease Control and Prevention, expansion of air surveillance efforts could provide additional safeguards for congregate settings and improve resilience to future respiratory virus threats[24].

Methods

Collection of air samples

AerosolSense instruments (Thermo Fisher Scientific, cat. 2900AA) were deployed in various indoor community settings for air pathogen surveillance. Samplers were placed in high-traffic areas on flat surfaces 2-5 feet off the ground and calibrated to sample 200 liters of air per minute. AerosolSense cartridges (Thermo Fisher Scientific, cat. 12148001) were installed and removed from the air sampler according to the manufacturer brochure and transferred to the lab for testing[61]. We developed a workflow to simplify data collection, management, and reporting (Figure 1). The workflow relies on the iOS and Android Askidd mobile app to easily collect air cartridge metadata and upload it to a centralized LabKey database. Air sampler users simply open the Askidd app, take a picture of the air cartridge barcode when installed and removed from the machine. The Askidd app collects GPS coordinates of the air sampler, timestamp, AerosolSense instrument ID, and air cartridge barcode to send to LabKey. When air sample testing was completed in the lab the results were uploaded to the Labkey database and displayed in the Askidd mobile app. This workflow tracks data for every cartridge and limits user errors that could occur during manual input.

Detection of SARS-CoV-2

University of Wisconsin-Madison

Hologic Aptima SARS-CoV-2 Assay

AerosolSense cartridges collected at a hospital in Dane County, Wisconsin from August 20th to October 25th, 2021 were tested for SARS-CoV-2 viral RNA using the Aptima SARS-CoV-2 Assay (Hologic) on the Panther System (Hologic). The Aptima SARS-CoV-2 Assay was authorized for emergency use authorization (EUA) by the United States Food and Drug Administration (FDA) for the qualitative detection of vRNA[62]. Air cartridges were collected from AerosolSense Samplers as recommended by the manufacturer. One substrate was removed from the cartridge using sterile forceps, transferred to a tube containing 750 µL of universal transport medium (Copan), and incubated at room temperature for 5-10 minutes. Following the incubation, 500 µL of the eluate was transferred to a Panther Fusion Specimen Lysis Tube (Hologic) containing 710 µL of specimen transport medium. The tube was gently mixed by inverting it several times before loading it into the Panther System to

automatically run the Aptima SARS-CoV-2 Assay as described by the manufacturer. Aptima SARS-CoV-2 positive and negative controls were run with each set of air samples. An estimated cut-off value of >650 RLU was used to consider samples as SARS-CoV-2 positive.

CDC SARS-CoV-2 RT-qPCR Assay

RNA extraction and real-time reverse-transcriptase polymerase chain reaction (RT-qPCR) testing of the air filter specimens occurred at the University of Wisconsin-Madison WVDL-WSLH COVID Laboratory (WWCL, Madison, Wisconsin). Each air cartridge was submerged in 500 µL of 1X PBS for at least one hour. For all air cartridges, the tubes were vortexed vigorously and 190 µL of the PBS was used for RNA extraction using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermo Fisher Scientific) on a 96-well KingFisher Flex extraction platform and eluted in a volume of 50 µL according to manufacturer's instructions. A multiplex one-step RT-qPCR assay targeting the 2019-nCoV N gene sequences (N1 and N2) and the human RnaseP (RP) gene was used for the SAR-CoV-2 viral detection. The RT-qPCR primers and probes sequences were based on the CDC assay with alternative fluorophores on the 5' end of each probe (along with 3' black hole quencher) for multiplexing[63]. The N1 probe was labeled with ABY dye, the N2 probe with the FAM dye, and the RP probe with the VIC dye. The 16 µL reaction mix consists of 1x TaqPath 1-Step Multiplex Master Mix (Thermo Fisher Scientific), 250 nM of each forward and reverse primers for the N1 and N2 targets, 100 nM of each forward and reverse primer for the RP gene target, 62.5 nM for each of the N1 and N2 probes, 50 nM of the RP probe and 5 ul of sample RNA or controls. The RT-qPCR amplification was performed with one cycle at 53°C for 10 mins and 95°C for 2 mins, followed by 40 cycles of 95°C for 3 secs and 60°C for 30 secs on a QuantStudio 7 Pro Real-Time PCR System (Thermo Fisher Scientific, Inc.). The data was analyzed in the Design and Analysis v2.4 software (Thermo Fisher Scientific, Inc.) using the auto baseline and threshold settings at 0.15 for N1 and N2 and 0.1 for the RP. Samples with amplification (Ct <40) in both the N1 and N2 targets were determined as positive for SARS-CoV-2. In contrast, samples with amplification in only 1 of the targets were determined as inconclusive, and samples without amplification in both N1 and N2 targets were deemed negative for SARS-CoV-2. Each run included a negative extraction control using a pool of previously identified SARS-CoV-2 negative samples, positive extraction control, negative template control, and positive amplification control plasmid. The RP gene was utilized for analysis of human nasal swab samples performed at the

same laboratory. It was not factored in for the result criteria for the air filter samples due to the low and inconsistent level of human cellular material trapped by the air filters.

TrueMark Respiratory Panel

AerosolSense cartridges collected from community testing sites from October 25, 2021 to February 9, 2022 were tested for the presence of 41 different respiratory tract viral, bacterial, and fungal nucleic acids using the TrueMark Respiratory Panel 2.0 TaqMan Array Card (TAC) (Thermo Fisher Scientific, Inc.). Substrates were extracted from the AerosolSense cartridge using sterile forceps, submerged into tubes containing 500 µL of PBS, vortexed for 5 seconds, and stored at 4C for 10-30 minutes. Samples were removed from 4C and sterile forceps were used to disrupt the substrate by pressing it against the bottom of the tube several times to ensure bound particles were eluted into the PBS. According to the manufacturer's recommendations, the substrate was removed from the tube and nucleic acids were isolated from the eluate using the Maxwell Viral Total Nucleic Acid Purification Kit (Promega) with the Maxwell 16 instrument (Promega). Briefly, 300 µL of the eluate was transferred to a tube containing 300 µL of lysis buffer and 30 µL of Proteinase K. A nuclease-free water control was processed with each Maxwell run and used in the TrueMark protocol as a no-template control. Tubes were vortexed for 5 seconds and incubated on a heat block at 56°C for 10 minutes. Following incubation, samples were centrifuged for 1 minute to pellet any debris. Then 630 µL of each reaction mix was transferred into a Maxwell 16 cartridge, loaded into Maxwell 16 instrument, and processed with the Viral Total Nucleic Acid program. Nucleic acids were eluted in 50 µL of RNase-free water. To perform the preamplification protocol, 5 µL of isolated nucleic acids were transferred into a PCR strip tube containing 2.5 µL of TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific, Inc.), and 2.5 µL of TrueMark Respiratory Panel 2.0 PreAmp Primers (Thermo Fisher Scientific, Inc.). Pre Amplification was performed on a thermocycler with the following cycling conditions: UNG incubation step at 25°C for 2 minutes, reverse transcription at 50°C for 30 minutes, UNG inactivation at 95°C for 2 minutes, 14 cycles at 95°C for 15 seconds (denaturation), 60°C for 2 minutes (annealing and extension), followed by inactivation at 99.9°C for 10 minutes, and 4°C until samples were ready for use. Preamplified products were diluted 1:20 in nuclease-free water, and the TrueMark Respiratory Panel 2.0 Amplification Control (Thermo Fisher Scientific, Inc.) was diluted 1:2 to include with every set of samples. TrueMark reaction mix was prepared by combining 20 µL of each diluted preamplified

product with 50 µL of TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Inc.) and 30 µL of nuclease-free water. TAC were equilibrated to room temperature, and 100 µL of each reaction mix was loaded into its respective TAC port. TAC were centrifuged twice at 1,200 rpm for 1 minute each spin. TAC were sealed with a TAC Sealer, loaded into the QuantStudio 7 Pro (QS7), and run with the settings recommended by the manufacturer. Data were exported from the QS7 into the Thermo Fisher Design and Analysis Software 2.6.0. Data were analyzed according to the manufacturer's recommendations using the relative quantification module with the relative threshold algorithm (Crt). Results were exported from the quality check module. Analysis was performed using a custom R script (v. 3.6.0) in RStudio (v. 1.3.959) to filter amplified results using the following cut-off values: amplification score >1.2 and Crt confidence >0.7. Samples were further filtered on reaction-specific Crt cut-off values determined in a limit-of-detection experiment (Supplementary Data 1). The TrueMark Respiratory Panel 2.0 includes technical control assays for human RNase P (RPPH1) and human 18S ribosomal RNA.

TrueMark Respiratory Panel Limit-of-Detection Estimation using Contrived Air Samples

Contrived air samples were prepared using the TrueMark Respiratory Panel 2.0 Amplification Control (Thermo Fisher Scientific, Inc.) to estimate the limit of detection (LOD) of the TrueMark Respiratory Panel 2.0 TaqMan Array Card. Briefly, two air cartridges were collected in an office for 48 hours each. The air cartridge substrates were processed and total nucleic acids were isolated as described above. Eluates from the four substrates were pooled together and aliquoted into five tubes. TrueMark amplification control plasmid, initially diluted in nuclease-free water, was added to four tubes at dilutions of 50 copies/µL, 10 copies/µL, 2.5 copies/µL, and 0.25 copies/µL. Final template concentrations for the preamplification reaction were 250, 50, 12.5, and 1.25 copies per reaction, respectively. No amplification control plasmid was added to the fifth tube that was used to determine the targets present in the background of contrived air samples collected from the empty office. An unused air cartridge was processed with the air samples as a negative template control. Four replicates of each contrived sample and control were processed through the reverse transcription, pre-amplification, dilution, and PCR protocols as described above. Data were analyzed in Thermo Fisher Design and Analysis Software 2.6.0 according to the manufacturer’s recommendations. Replicates were called positive using the following cut-off values: amplification score >1.2 and Crt confidence >0.7. Cycle relative threshold cut-off values were determined by averaging the Crt values of positive

replicates at the lowest dilution concentration with at least 75% positive replicates. Any reaction targets that were detected in the contrived air sample or not detected at the highest amplification control dilution were excluded from the analysis and Crt cut-off values defaulted to the manufacturer's recommendation of Crt >30.

University of Minnesota

Nucleic Acid Extraction and RT-qPCR

To elute the sample from the AerosolSense cartridges, both substrates were placed into 1mL of PBS, making sure the substrates were fully saturated with PBS. A pipette was used to push down on the substrates to extract as much eluate out of them as possible. Eluate was then transferred to a new tube. Samples were extracted using the *Quick*-RNA Viral Kit (Zymo Research). The extraction method followed manufacturer-recommended protocols with the notable exceptions of using 100 µL of starting material and eluting with 65 µL of appropriate elution material as indicated by manufacturer protocols. RT-qPCR reactions were set up in a 96-well Barcoded plate (Thermo Scientific) for either the N1 or N2 primers and probes with CDC-recommended sequences[64]. Then 5 µL extracted RNA was added to 15 µL qPCR master mix comprised of the following components: 8.5 µL nuclease-free water, 5 µL TaqMan™ Fast Virus 1-Step Master Mix (Thermo), and 1.5 µL primer/probe sets for either N1 or N2 (IDT, Cat# 10006713). SARS-CoV-2 RNA Control was obtained from Twist Biosciences (Genbank Ref. No. MN908947.3) and used as a positive control in each run. Reactions were cycled in a QuantStudio QS3 (ThermoFisher) for one cycle of 50°C for 5 minutes, followed by one cycle of 95°C for 20 seconds, followed by 50 cycles of 95°C for 3 seconds and 55°C for 30 seconds. A minimum of two no-template controls (NTCs) were included on all runs. Baselines were allowed to calculate automatically, and a ΔRn threshold of 0.5 was selected and set uniformly for all runs. Ct values were exported and analyzed in Microsoft Excel. Amplification curves were manually reviewed. Samples with Ct<40 in both N1 and N2 reactions were determined as positive for SARS-CoV-2. In contrast, samples with Ct<40 in only N1 or N2 targets were determined as inconclusive results, and samples without amplification in both N1 and N2 targets were deemed negative for SARS-CoV-2.

SARS-CoV-2 Spike Receptor Binding Domain Sequencing

Targeted sequencing of SARS-CoV-2 spike receptor-binding domain (RBD) was performed as previously described[26]. The data were analyzed using a custom workflow implemented in Snakemake[65]. Briefly, paired-end reads were interleaved and merged into synthetic reads spanning the entire RBD PCR amplicon using bbmerge.sh (v38.93) from the bbtools package (sourceforge.net/projects/bbmap/) with default parameters. The merged reads were mapped to the SARS-CoV-2 reference sequence (Genbank MN908947.3) using minimap2 (v2.24) with the `-ax sr` preset for short reads. The resulting mapping file was sorted with samtools (v1.14). Reads that fully contain the desired amplicon sequence were extracted with the bedtools (v2.30.0) intersect tool. These reads were then downsampled to a target depth of 1000 reads using reformat.sh (v38.93) from the bbtools package. These downsampled reads were remapped to the MN908947.3 reference with minimap2. Residual PCR primer sequences were then trimmed with samtools ampliconclip using the `--hard-clip --both-ends` parameters. Next, a consensus sequence was generated by first generating a pileup with the samtools mpileup tool using default settings and then generating a consensus with ivar (v1.3.1) using the parameters `-q 20 -t 0 -m 20`.

At the same time a consensus sequence was generated, the primer-trimmed reads were deduplicated to determine how many of the reads were identical, essentially defining pseudo-haplotypes. Vsearch (v2.21.1) fastx_uniques tool was used for deduplicating and enumerating the number of identical reads in each sample.

Lineage-defining mutations in the RBD were used to differentiate Delta from Omicron consensus sequences. Only one sample had evidence of mixed Delta and Omicron sequences.

City of Milwaukee Health Department

Nucleic Acid Extraction and RT-qPCR

Upon receipt of Thermo Scientific AerosolSense 2900 air sampler cartridge at City of Milwaukee Health Department Laboratory (MHDL), collection substrates were aseptically removed and transferred to a 5 mL sterile screw-cap tube filled with 1 mL of Remel viral transport medium. Samples were kept frozen at -70°C until total nucleic extraction was performed using 200 µL elute and Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit using ThermoFisher Scientific

KingFisher Flex instrument. Real-time RT-PCR setup was performed using 10 µL of extract and approved Applied Biosystems TaqPath™ COVID-19 Combo Kit containing three primer/probe sets specific to different SARS-CoV-2 genomic regions (open reading frame 1ab (ORF1ab), spike (S) protein, and nucleocapsid (N) protein-encoding genes) and primers/probes for bacteriophage MS2 which served as internal process control for nucleic acid extraction. RT-PCR assay was performed on Applied Biosystems 7500 Fast Dx Real-Time PCR System according to the TaqPath™ COVID-19 Combo Kit protocol. PCR results were interpreted using the Applied Biosystems COVID-19 Interpretive Software, and results were reported as positive, negative, or inconclusive for the detection of SARS-CoV-2 RNA following successful quality control checks.

SARS-CoV-2 Sequencing

Samples with Ct values below 30 were sequenced using the ARTIC protocol and the Illumina DNA Prep library kit on a MiSeq instrument (https://www.protocols.io/view/sars-cov-2-sequencing-on-illumina-miseq-using-arti-bssjnecn). Data generated using the Integrated DNA Technologies ARCTIC V4 primer panel were analyzed using the Illumina® DRAGEN COVID Lineage App, which uses a customized version of the DRAGEN DNA pipeline to perform Kmer-based detection of SARS-CoV-2. The app aligns reads to a reference genome, calls variants, and generates a consensus genome sequence. Lineage/clade assignments were also confirmed using NextClade (https://clades.nextstrain.org/, version 1.14.0) and Pangolin COVID-19 Lineage Assigner (https://pangolin.cog-uk.io/, version 3.1.20) by uploading obtained FASTA files [66,67]. Consensus sequences generated and related metadata for environmental samples were shared publicly on Global Initiative on Sharing All Influenza Data (GISAID) (www.gisaid.org), the principal repository for SARS-CoV-2 genetic information.

Mayo Clinic

Nucleic Acid Extraction and RT-qPCR

Upon removal of the screw cap from the air sample cartridge in the biosafety level 2 cabinet, the air cartridge substrate was removed with a pair of disposable sterile forceps and transferred into a sterile tube containing 1 mL of phosphate-buffered saline (PBS). The tube was vortexed for 10 sec, and 200µL of the sample was used for nucleic acid extraction and purification on the KingFisher Flex magnetic particle processor (Thermo Fisher Scientific, Inc.) using the MagMAX Viral/Pathogen II

Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Inc.) and MVP_Flex_200ul software program, with an elution volume of 50 uL. SARS-CoV-2 sequence targets (ORF1ab, N, and S gene sequences) were amplified and detected with the FDA-authorized TaqPath COVID-19 Combo Kit (Life Technologies Corp., Pleasanton, CA) on the Applied Biosystems 7500 Fast Dx Real-Time PCR System (Life Technologies Corp.) per assay manufacturer's instructions for use.

Ethics statement

The Institutional Review Board of the University of Wisconsin-Madison Health Sciences waived ethical approval of this work.

References

1. Lee, J. S. et al. Analysis of the initial lot of the CDC 2019-Novel Coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel. PLoS One **16**, e0260487 (2021).

2. Wu, K. J. 'It's Like Groundhog Day': Coronavirus Testing Labs Again Lack Key Supplies. The New York Times (2020).

3. Supply shortages impacting COVID-19 and non-COVID testing. ASM.org https://asm.org/Articles/2020/September/Clinical-Microbiology-Supply-Shortage-Collecti-1 (2021).

4. Vogels, C. B. F. et al. SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. Med (N Y) **2**, 263–280.e6 (2021).

5. CDC. COVID data tracker. Centers for Disease Control and Prevention https://covid.cdc.gov/covid-data-tracker/?ACSTrackingID=FCP_1_USCDC_2145-DM75072&ACSTrackingLabel=%5BProof%201%5D%2002.04.2022%20-%20COVID-19%20Data%20Tracker%20Weekly%20Review&deliveryName=FCP_1_USCDC_2145-DM75072 (2020).

6. Da Silva, C., Murphy, J. & Lovelace, B., Jr. U.S. reports record 1.3M Covid cases in a day as hospitalizations soar. NBC News https://www.nbcnews.com/news/us-news/us-reports-record-13m-covid-cases-day-hospitalizations-soar-rcna11736 (2022).

7. Kalish, H. et al. Undiagnosed SARS-CoV-2 seropositivity during the first 6 months of the COVID-19 pandemic in the United States. Sci. Transl. Med. **13**, (2021).

8. Fact sheet: The Biden Administration to begin distributing at-home, rapid COVID-19 tests to Americans for Free. The White House https://www.whitehouse.gov/briefing-room/statements-releases/2022/01/14/fact-sheet-the-biden-administration-to-begin-distributing-at-home-rapid-covid-19-tests-to-americans-for-free/ (2022).

9. Rubin, R. First It Was Masks; Now Some Refuse Testing for SARS-CoV-2. JAMA **324**, 2015–2016 (2020).

10. News Division. Biden administration to invest more than $12 billion to expand COVID-19 testing. HHS.gov https://www.hhs.gov/about/news/2021/03/17/biden-administration-invest-more-than-12-billion-expand-covid-19-testing.html (2021).

11. Larremore, D. B. et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv **7**, (2021).

12. Paltiel, A. D., Zheng, A. & Walensky, R. P. Assessment of SARS-CoV-2 Screening Strategies to Permit the Safe Reopening of College Campuses in the United States. JAMA Netw Open **3**, e2016818 (2020).

13. Chin, E. T. et al. Frequency of Routine Testing for Coronavirus Disease 2019 (COVID-19) in High-risk Healthcare Environments to Reduce Outbreaks. Clin. Infect. Dis. **73**, e3127–e3129 (2021).

14. Mina, M. J., Parker, R. & Larremore, D. B. Rethinking covid-19 test sensitivity - A strategy for containment. N. Engl. J. Med. **383**, e120 (2020).

15. Newman, C. M. et al. Initial Evaluation of a Mobile SARS-CoV-2 RT-LAMP Testing Strategy. J. Biomol. Tech. **32**, 137–147 (2021).

16. Denny, T. N. et al. Implementation of a Pooled Surveillance Testing Program for Asymptomatic SARS-CoV-2 Infections on a College Campus - Duke University, Durham, North Carolina, August 2-October 11, 2020. MMWR Morb. Mortal. Wkly. Rep. **69**, 1743–1747 (2020).

17. Mukherjee, U. K. et al. Author Correction: Evaluation of reopening strategies for educational institutions during COVID-19 through agent based simulation. Sci. Rep. **11**, 20667 (2021).

18. Holden, T. M. et al. Geographic and demographic heterogeneity of SARS-CoV-2 diagnostic testing in Illinois, USA, March to December 2020. BMC Public Health **21**, 1105 (2021).

19. Grigsby-Toussaint, D. S., Shin, J. C. & Jones, A. Disparities in the distribution of COVID-19 testing sites in black and Latino areas in new York City. Prev. Med. **147**, 106463 (2021).

20. Cordes, J. & Castro, M. C. Spatial analysis of COVID-19 clusters and contextual factors in New York City. Spat. Spatiotemporal Epidemiol. **34**, 100355 (2020).

21. Tao, R., Downs, J., Beckie, T. M., Chen, Y. & McNelley, W. Examining spatial accessibility to COVID-19 testing sites in Florida. Ann. GIS **26**, 319–327 (2020).

22. Credit, K. Neighbourhood inequity: Exploring the factors underlying racial and ethnic disparities in COVID-19 testing and infection rates using ZIP code data in Chicago and New York. Reg. sci. policy pract. **12**, 1249–1271 (2020).

662 23. Rader, B. et al. Geographic access to United States SARS-CoV-2 testing sites highlights
663 healthcare disparities and may bias transmission estimates. J. Travel Med. **27**, (2020).

664 24. CDC. National Wastewater Surveillance System (NWSS). Centers for Disease Control and
665 Prevention https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-
666 surveillance.html (2022).

667 25. Harris-Lovett, S. et al. Wastewater Surveillance for SARS-CoV-2 on College Campuses: Initial
668 Efforts, Lessons Learned, and Research Needs. Int. J. Environ. Res. Public Health **18**, (2021).

669 26. Gregory, D. A., Wieberg, C. G., Wenzel, J., Lin, C.-H. & Johnson, M. C. Monitoring SARS-CoV-2
670 Populations in Wastewater by Amplicon Sequencing and Using the Novel Program SAM Refiner.
671 Viruses **13**, (2021).

672 27. Peccia, J. et al. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection
673 dynamics. Nat. Biotechnol. **38**, 1164–1167 (2020).

674 28. Ahmed, W. et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia:
675 A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total
676 Environ. **728**, 138764 (2020).

677 29. San Diego wastewater surveillance. SEARCH https://searchcovid.info/dashboards/wastewater-
678 surveillance/ (2021).

679 30. Xu, Y. et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for
680 persistent fecal viral shedding. Nat. Med. **26**, 502–505 (2020).

681 31. Chen, Y. et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J. Med. Virol.
682 **92**, 833–840 (2020).

683 32. Zheng, S. et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2
684 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ **369**, m1443
685 (2020).

686 33. Yang, W., Elankumaran, S. & Marr, L. C. Concentrations and size distributions of airborne influenza
687 A viruses measured indoors at a health centre, a day-care centre and on aeroplanes. J. R. Soc.
688 Interface **8**, 1176–1184 (2011).

689 34. Prussin, A. J., 2nd & Marr, L. C. Sources of airborne microorganisms in the built environment.
690 Microbiome **3**, 78 (2015).

691 35. Wang, C. C. et al. Airborne transmission of respiratory viruses. Science **373**, (2021).

692 36. Myatt, T. A. et al. Detection of airborne rhinovirus and its relation to outdoor air supply in office
693 environments. Am. J. Respir. Crit. Care Med. **169**, 1187–1190 (2004).

694 37. Institute of Medicine (US), National Research Council (US) Committee on Effectiveness of National
695 Biosurveillance Systems: Biowatch & the Public Health System. The BioWatch System. (National
696 Academies Press (US), 2011).

697 38. Horve, P. F., Dietz, L., Northcutt, D., Stenson, J. & Van Den Wymelenberg, K. Evaluation of a
698 bioaerosol sampler for indoor environmental surveillance of Severe Acute Respiratory Syndrome
699 Coronavirus 2. PLoS One **16**, e0257689 (2021).

700 39. Dietz, L. et al. Exploring Integrated Environmental Viral Surveillance of Indoor Environments: A
701 comparison of surface and bioaerosol environmental sampling in hospital rooms with COVID-19
702 patients. bioRxiv (2021) doi:10.1101/2021.03.26.21254416.

703 40. Parhizkar, H. et al. Quantifying human and environmental viral load relationships amidst mitigation
704 strategies in a controlled chamber with participants having COVID-19. Research Square (2021)
705 doi:10.21203/rs.3.rs-940891/v1.

706 41. Horve, P. et al. Longitudinal analysis of built environment and aerosol contamination associated
707 with isolated COVID-19 positive individuals. Research Square (2021) doi:10.21203/rs.3.rs-861942/
708 v1.

709 42. Lednicky, J. A. et al. Collection of SARS-CoV-2 Virus from the Air of a Clinic Within a University
710 Student Health Care Center and Analyses of the Viral Genomic Sequence. Aerosol Air Qual. Res.
711 **20**, 1167–1171 (2020).

712 43. Santarpia, J. L. et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine
713 and isolation care. Sci. Rep. **10**, 12732 (2020).

714 44. TrueMark respiratory panel 2.0 - US.

45. Kahn, J. S. et al. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. J. Infect. Dis. **198**, 41–50 (2008).

46. Wisconsin DHS Health Alert #35: Increased Influenza Identified in Midwest Universities. Wisconsin Department of Health Services https://content.govdelivery.com/accounts/WIDHS/bulletins/2fe8698.

47. Smyth, D. S. et al. Tracking cryptic SARS-CoV-2 lineages detected in NYC wastewater. Nat. Commun. **13**, 635 (2022).

48. COVID-19: Wisconsin Coronavirus wastewater monitoring network. Wisconsin Department of Health Services https://www.dhs.wisconsin.gov/covid-19/wastewater.htm (2020).

49. Governor Evers, DHS announce more than $175 million to support COVID-19 testing in schools. Wisconsin Department of Health Services https://www.dhs.wisconsin.gov/news/releases/042021.htm (2021).

50. Wisconsin Schools. https://www.greatschools.org/wisconsin/.

51. Wisconsin child care centers. https://childcarecenter.us/state/wisconsin.

52. CMS changes Medicare payment to support faster COVID-19 diagnostic testing. https://www.cms.gov/newsroom/press-releases/cms-changes-medicare-payment-support-faster-covid-19-diagnostic-testing.

53. Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of Concern. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern.

54. Lednicky, J. A. et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. Int. J. Infect. Dis. **100**, 476–482 (2020).

55. Fabian, P., McDevitt, J. J., Houseman, E. A. & Milton, D. K. Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: considerations for a new infectious virus aerosol sampler. Indoor Air **19**, 433–441 (2009).

56. Pan, M., Lednicky, J. A. & Wu, C.-Y. Collection, particle sizing and detection of airborne viruses. J. Appl. Microbiol. **127**, 1596–1611 (2019).

57. Haktanir, A., Can, N., Seki, T., Kurnaz, M. F. & Dilmaç, B. Do we experience pandemic fatigue? current state, predictors, and prevention. Curr. Psychol. 1–12 (2021) doi:10.1007/s12144-021-02397-w.

58. Riemersma, K. K. et al. Shedding of infectious SARS-CoV-2 despite vaccination. bioRxiv (2021) doi:10.1101/2021.07.31.21261387.

59. Andrews, N. et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. N. Engl. J. Med. (2022) doi:10.1056/NEJMoa2119451.

60. Galani, A. et al. SARS-CoV-2 wastewater surveillance data can predict hospitalizations and ICU admissions. Sci. Total Environ. **804**, 150151 (2022).

61. AerosolSense™ Sampler. https://www.thermofisher.com/order/catalog/product/AEROSOLSENSE?SID=srch-srp-AEROSOLSENSE.

62. Aptima SARS-CoV-2 assay - Letter of Authorization. https://www.fda.gov/media/138097/download.

63. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. https://www.fda.gov/media/134922/download.

64. 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes. https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf.

65. Mölder, F. et al. Sustainable data analysis with Snakemake. F1000Res. **10**, 33 (2021).

66. Aksamentov, I., Roemer, C., Hodcroft, E. & Neher, R. Nextclade: clade assignment, mutation calling and quality control for viral genomes. J. Open Source Softw. **6**, 3773 (2021).

67. O'Toole, Á. et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. Virus Evol **7**, veab064 (2021).

Acknowledgements

This work was made possible by financial support through Rockefeller Regional Accelerator for Genomic Surveillance (133 AAJ4558), Wisconsin Department of Health Services Epidemiology and Laboratory Capacity funds (144 AAJ8216), and National Institutes of Health grant (U01DA053893). We would like to thank all of the participating congregate settings for their partnership during this study. We would like to thank Thermo Fisher Scientific, Inc. for donating 4 of the AerosolSense samplers used in the data described in this work. We would like to acknowledge Elizabeth Durkes and Hannah Kraussel, Public Health Emergency Response Planning Coordinators, Lindor Schmidt, Environmental & Disease Control Specialist, and Dr. Nicholas Tomaro, Emergency Preparedness Environmental Health Director at the City of Milwaukee Health Department for their assistance in coordinating site selection, sampling, cartridge replacement, and unit decontamination processes.

Contributions

M.D.R contributed to the conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing—original draft preparation, writing—review and editing. C.M.N. contributed to data curation, investigation, methodology, supervision, writing—review and editing. S.F.B. contributed to project administration, data curation, resources, visualization, writing—review and editing. M.R.S. contributed to the investigation, data curation, methodology, writing—review and editing. R.W.W. contributed to data curation, methodology, writing—review and editing. A.K.Y. contributed to resources, writing—review and editing. E.J.O. contributed to software, data curation, writing—review and editing. N.D. contributed to the investigation, data curation, methodology, writing—review and editing. A.L. contributed to the investigation, formal analysis, data curation, methodology, supervision, writing—review and editing. K.P.P. contributed to resources, supervision, writing—review and editing. N.S. contributed to resources, supervision, data curation, writing—review and editing. J.A.M. contributed to resources, supervision, data curation, writing—review and editing. M.A.A. contributed to resources, supervision, data curation, writing—review and editing. W.M.R contributed to resources, supervision, data curation, writing—review and editing. J.A.Z. Contributed to the investigation, developed methods, sample processing, performed analysis, data curation, writing—review and editing. M.K. Contributed to the method development, analysis, data curation, writing—review and editing. L.J.B. contributed to conceptualization, data curation, formal analysis, investigation, methodology, writing—review and editing. E.C.B. contributed to the investigation, formal analysis, data curation, methodology, writing—review and editing. D.C. contributed to the investigation, formal analysis, data curation, methodology, writing—review and editing. C.R. contributed to the investigation, data curation, methodology, writing—review and editing. D.A.G contributed to formal analysis, data curation, writing—review and editing. J.D.Y. contributed to conceptualization, supervision, methodology, writing—review and editing. S.B. contributed to resources, conceptualization, supervision, writing—review and editing. M.C.J. contributed to the investigation, data curation, methodology, writing—review and editing. M.T.A. contributed to conceptualization, supervision, methodology, writing—review and editing. T.C.F. contributed to the conceptualization, funding acquisition, writing—review and editing. D.H.O. contributed to the conceptualization, formal analysis, software, funding acquisition, methodology, supervision, project administration, writing—original draft preparation, writing—review and editing. S.L.O. contributed to the conceptualization, funding acquisition, methodology, supervision, project administration, writing—review and editing.

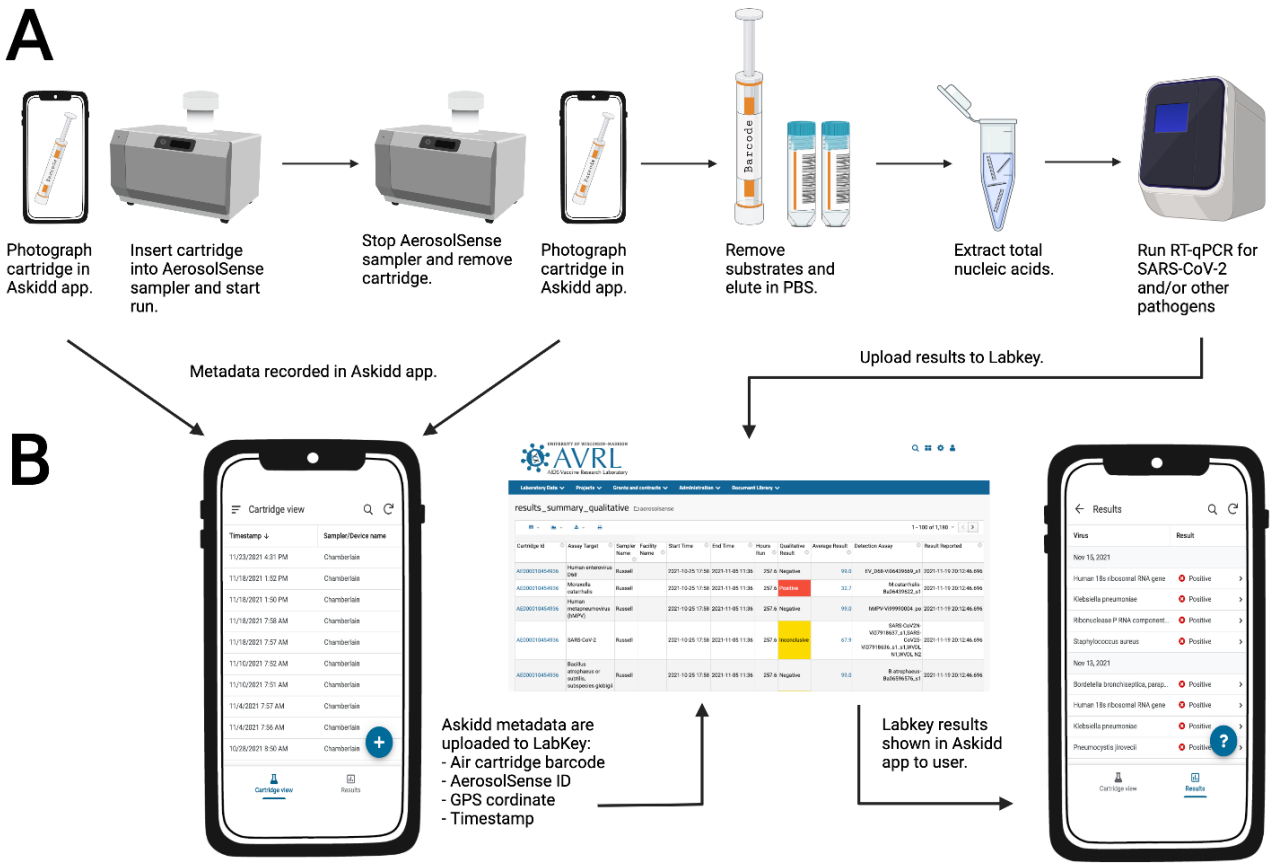

Figure 01. Air sample testing workflow. (A) Overview of air sample collection, processing, and testing. (B) Air sample data collection and management. Individuals in charge of changing air cartridges at surveillance sites use the iOS and Android Askidd mobile app to collect metadata on air samples when cartridges are inserted and removed. Data are compiled in Labkey database and displayed to surveillance sites in the Askidd mobile app. Created with BioRender.com.

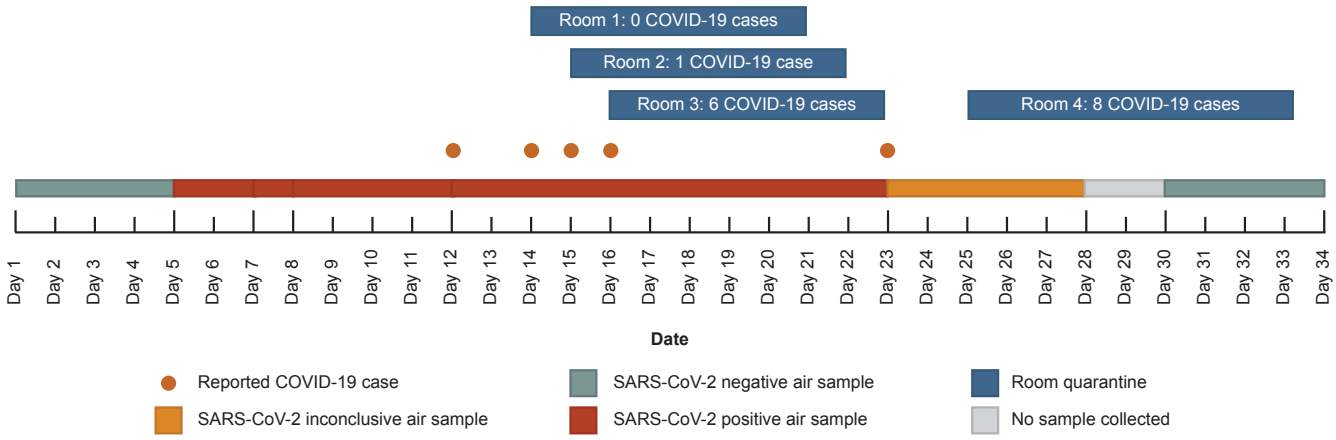

Figure 02. COVID-19 outbreak timeline. Confirmed COVID-19 cases and air sample SARS-CoV-2 RT-qPCR results in the congregate setting are plotted over time. Orange dots represent confirmed COVID-19 cases from individuals present in the building. Blue boxes show the number of COVID-19 cases that occurred while close contacts were in quarantine. Air sample SARS-CoV-2 RT-qPCR results are represented by boxes as positive (green), negative (red), or inconclusive (orange). The gray box indicates that no sample was collected during that time period.

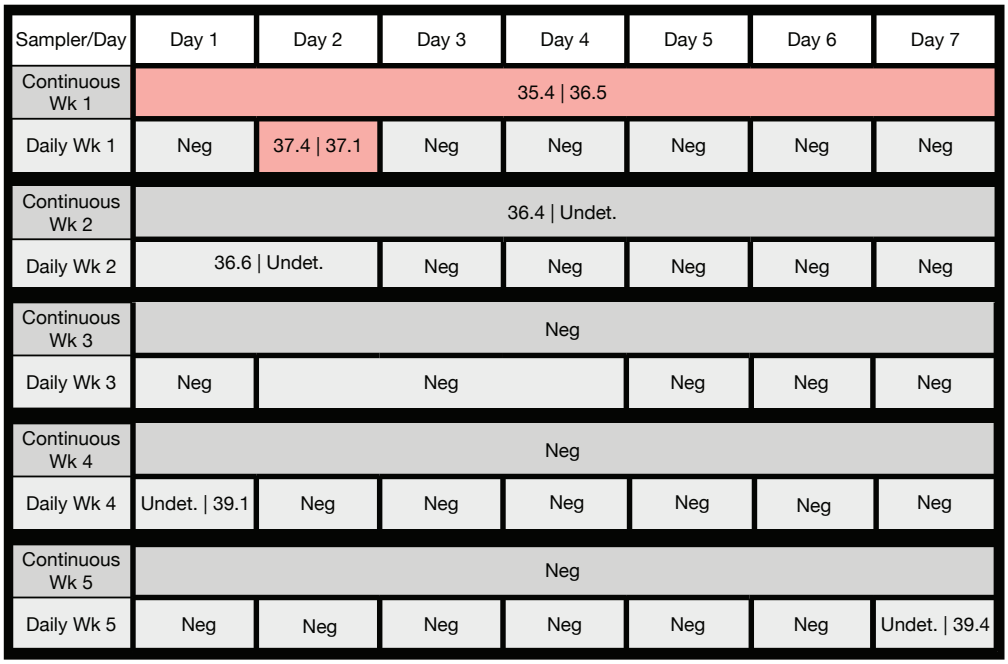

Figure 03. Comparison of SARS-CoV-2 RT-qPCR results from continuous and daily air sampling intervals. Two adjacent Thermo Scientific AerosolSense instruments were run continuously or daily over several days. SARS-CoV-2 genomic material was detected by two separate RT-qPCR CDC assays. If the cycle threshold values of one or both N1 and N2 assays were less than 40, the Ct values are shown in the box separated by a '|'. 'Undet.' was used for assays that had Ct values greater than 40. If the Ct values of both RT-qPCR assays were greater than 40, the boxes are labeled as 'Neg'. Samples considered to be positive are shaded red. Boxes shaded in grey are either inconclusive or negative.

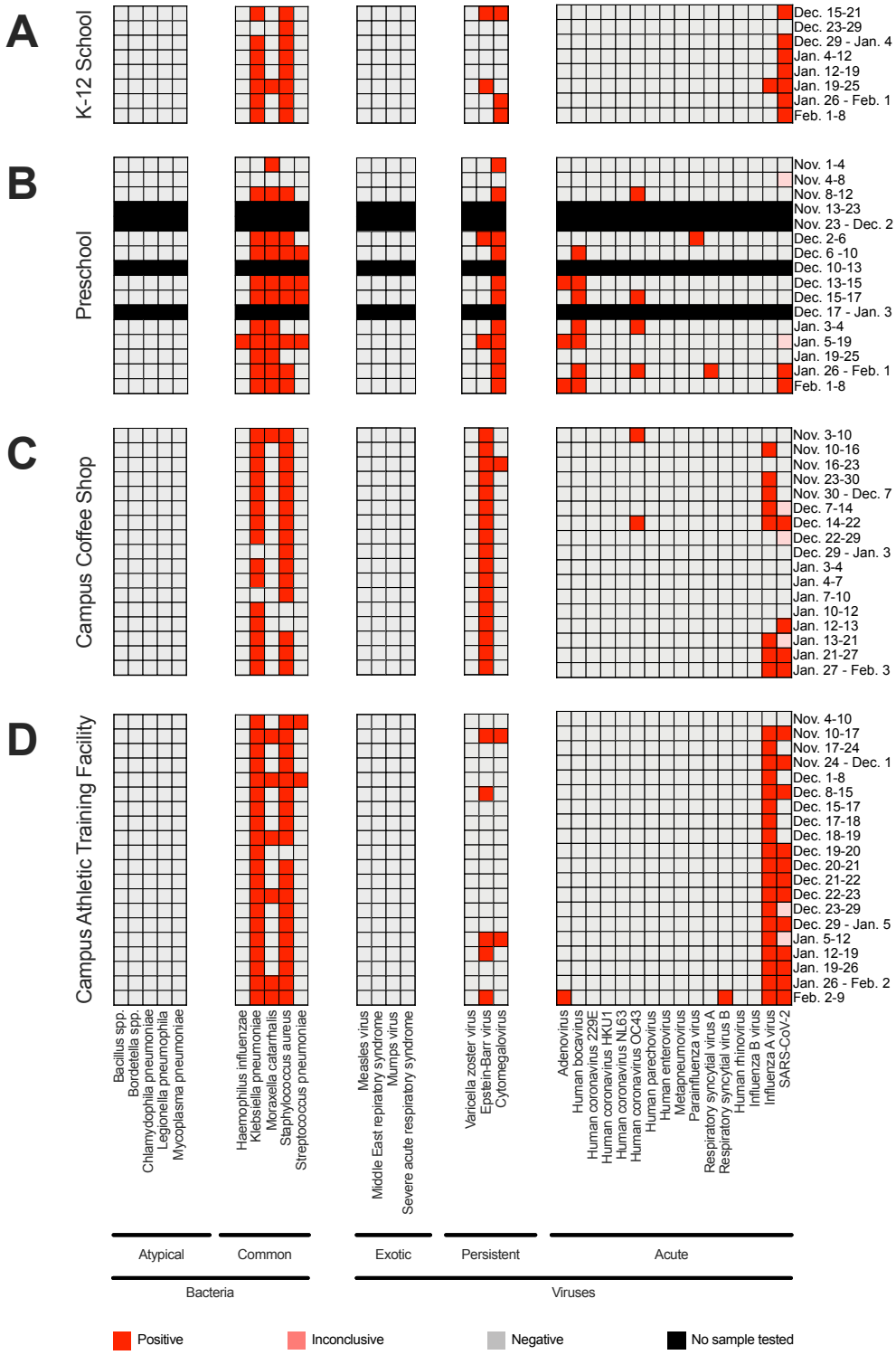

Figure 04. In-air respiratory pathogen detection in congregate settings. (A) Respiratory pathogen detection in air samples collected from a K-12 school, (B) preschool, (C) campus coffee shop, and (D) campus athletic facility. Genomic material from 40 respiratory pathogens was detected by semi-quantitative RT-PCR using the TrueMark Respiratory 2.0 TaqMan Array Card. SARS-CoV-2 genomic material was detected by two separate RT-qPCR CDC assays. Boxes shaded in red, pink, and gray represent positive, inconclusive, and negative air samples collected during the sampling interval on the x-axis. No sample was tested for boxes shaded in black.

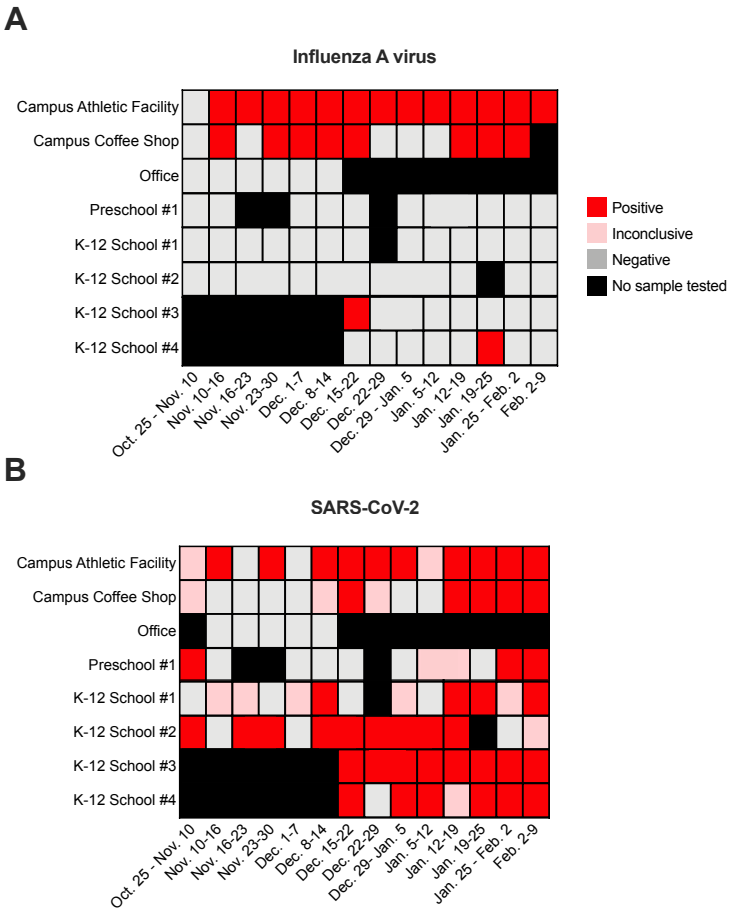

Figure 05. Detection of SARS-CoV-2 and influenza A virus in Dane County, WI. (A) Influenza A virus (IAV) detection in air samples collected from congregate settings. IAV genomic material was detected by semi-quantitative RT-PCR using the TrueMark Respiratory 2.0 TaqMan Array Card. (B) SARS-CoV-2 detection in air samples collected from congregate settings. SARS-CoV-2 genomic material was detected by two separate RT-qPCR N1 and N2 CDC assays. Boxes shaded in red, pink, and gray represent positive, inconclusive, and negative air samples collected during the sampling interval in the x-axis. No sample was tested for boxes shaded in black. Campus sites were located on the college campus of the University of Wisconsin-Madison.

Table 1. SARS-CoV-2 air sample results.

Location	Site Name	Start Date	End Date	Number of Samples	Positive	Negative	Inconclusive
Dane County, WI	Preschool #1	8/18/21	2/8/22	49	3	43	3
	Preschool #2	8/11/21	10/14/21	22	2	18	2
	K-12 School #1	7/26/21	2/8/22	73	4	62	7
	K-12 School #2	10/14/21	2/9/22	15	8	5	2
	K-12 School #3	12/14/21	2/8/22	7	7	0	0
	K-12 School #4	12/15/21	2/8/22	8	6	1	1
	Hospital	8/20/21	10/25/21	51	18	33	0
	Campus Athletic Facility	7/19/21	2/9/22	179	20	141	18
	Campus Coffee Shop	8/17/21	2/3/22	54	5	44	5
	Office	9/30/21	12/10/21	8	0	8	0
Minneapolis, MN	Brewery taproom	10/18/21	2/7/22	26	11	2	13
Rochester, MN	Bar	9/27/21	11/24/21	9	5	4	0
	Hospital Cafeteria	9/20/21	11/24/21	10	6	4	0
Milwaukee, WI	Emergency Housing Facility #1	12/17/21	2/8/22	9	5	3	1
	Emergency Housing Facility #2	12/17/21	2/8/22	7	6	1	0
			Total	**527**	**106**	**369**	**52**

Table 2. Air sample SARS-CoV-2 sequencing results.

Location	Air sample barcode	Start	Finish	SARS-CoV-2 N gene PCR Ct	Lineage(s)	Spike RBD amino acid differences vs. SARS-CoV-2 reference	Accession Number
Emergency Housing Facility #1	AE000010795F4A	12/21/21	1/7/22	31.77	BA.1	S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	EPI_ISL_8879389
Emergency Housing Facility #2	AE000010795B42	12/21/21	1/7/22	25.94	BA.1	S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	EPI_ISL_8879388
Brewery taproom	AE000010466C36	11/22/21	11/29/21	30.5 | 34.28	Delta	L452R; T478K	SRX14331279
Brewery taproom	AE000010464938	11/22/21	11/29/21	31.63 | 35.15	Delta	L452R; T478K	SRX14331280
Brewery taproom	AE000010467837	12/6/21	12/13/21	38 | 69.63	Delta	L452R; T478K	SRX14331281
Brewery taproom	AE000010467A3C	12/6/21	12/13/21	37.41 | 42.9	Delta	L452R; F456L; T478K; F562F	SRX14331282
Brewery taproom	AE000010463B32	12/30/21	1/3/22	35.28 | 38.77	BA.1	K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	SRX14331283
Brewery taproom	AE00001046442E	12/30/21	1/3/22	35.69 | 37.9	Delta and BA.1	K417N; N440K; G446S; L452R; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	SRX14331284
Brewery taproom	AE000010463F3A	1/3/22	1/10/22	33.85 | 37.47	BA.1	K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	SRX14331285
Brewery taproom	AE000010465530	1/10/22	1/17/22	33.37 | 36.34	BA.1	K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	SRX14331286
Brewery taproom	AE00001053FA3D	1/17/22	1/25/22	34.04 | 38.94	BA.1	K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K	SRX14331287

Samples with two cycle threshold (Ct) values listed in the table were tested with two SARS-CoV-2 N1 and N2 assays. N1 and N2 Ct values are separated by '|'. Samples with one Ct value listed in the table were tested with the Applied Biosystems TaqPath™ COVID-19 Combo Kit. Abbreviations: RBD, receptor binding domain; Ct, cycle threshold.