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Detection of SARS-CoV-2 RNA on air purifier filters in university spaces without symptomatic or confirmed cases

Jing Li^a, Merel Bot^b, Xinlei Liu^c, Yuan Yao^a, Roel A. Ophoff^b, and Yifang Zhu^a

^aDepartment of Environmental Health Sciences, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, USA; ^bCenter for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, University of California Los Angeles, Los Angeles, California, USA; ^cDepartment of Environmental Studies, New York University, New York, New York, USA

ABSTRACT

Understanding the seasonality and prevalence of respiratory viruses in indoor environments is essential for protecting public health in the post-pandemic era. This study investigated the presence of airborne SARS-CoV-2 in university indoor spaces where no symptomatic or confirmed positive individuals were supposed to be present. A total of 127 high-efficiency particulate air (HEPA) filter samples were analyzed from air purifiers installed in classrooms, conference rooms, and a community room within a university building across different seasons from Fall 2022 to Summer 2023. Viral RNA was extracted and quantified using RT-qPCR for each sample. SARS-CoV-2 RNA was detected in 21% of the samples, with the positivity rate varying significantly by room type but not by season. Among the 27 positive samples, viral RNA concentrations were significantly higher in fall-winter compared to summer, with no significant differences across room types. Additionally, respiratory syncytial virus (RSV) and influenza A virus (IAV) were detected in far fewer samples (positive rates: 2% and 4%, respectively) and at much lower concentrations than SARS-CoV-2. These findings provide evidence of the potential for airborne SARS-CoV-2 transmission in shared indoor spaces, even in the absence of known infectious individuals. They also suggest that SARS-CoV-2 may circulate in each season, underscoring the continued need for interventions to reduce indoor viral exposure.

ARTICLE HISTORY

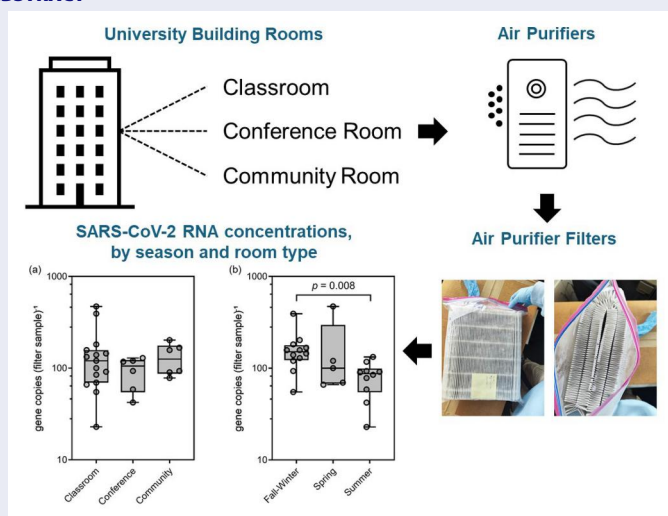
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GRAPHICAL ABSTRACT



CONTACT Yifang Zhu yifang@ucla.edu Department of Environmental Health Sciences, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA 90095, United States.

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Introduction

Virus-laden aerosols are a primary mode of COVID-19 transmission over both short and long distances, posing serious respiratory health risks to the public. This mode of transmission was acknowledged by the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) in 2021 (Wang et al. 2021). Numerous studies have detected SARS-CoV-2 in indoor air samples, heating, ventilation, and air conditioning (HVAC) filters, and air purifier high-efficiency particulate air (HEPA) filters, where elevated concentrations of SARS-CoV-2 are expected. Most of these studies were conducted in healthcare facilities (Chia et al. 2020; Guo et al. 2020; Liu et al. 2020; Mouchtouri et al. 2020; Nissen et al. 2020; Horve et al. 2021; Fernández de Mera et al. 2022; Groma et al. 2023), quarantine or isolation rooms (Santarpia et al. 2020; Pan et al. 2022), and non-healthcare settings such as homes with confirmed positive cases (Mouchtouri et al. 2020; Maestre et al. 2021; Rodríguez et al. 2021; Correia et al. 2022; Myers et al. 2022; Shankar et al. 2022; Sousan et al. 2022). Fewer studies have examined non-healthcare environments with asymptomatic populations, such as schools and university spaces (Zuniga-Montanez et al. 2022; Hoskin et al. 2024; Wang et al. 2025) or child daycare centers (Clarke et al. 2023). The COVID-19 public health emergency officially ended in May 2023, as declared by both the WHO (WHO 2023) and the U.S. government (Hodge 2023). Since then, very few studies (Wang et al. 2025) have explored the presence of airborne SARS-CoV-2 in non-healthcare settings without known infectious individuals, either symptomatic or confirmed positive, in the post-pandemic period.

Moreover, in the late- and post-pandemic, the co-circulation of respiratory viruses shifted dramatically compared to patterns observed during the COVID-19 pandemic. At the onset of the pandemic, infections caused by respiratory viruses such as influenza and respiratory syncytial virus (RSV) declined sharply and remained low to varying degrees during subsequent waves of SARS-CoV-2 infections (Chow et al. 2023). However, in the fall and winter of 2022, pediatric hospital units across the United States faced unprecedented strain from a “triple-demic”—a surge in respiratory diseases that included RSV infections in children, a sharp rise in influenza cases, and the continued circulation of COVID-19 (Furrow 2023). According to a survey conducted by the Kaiser Family Foundation between January 17 and 24, 2023, nearly 40% of households reported being affected by the “triple-demic” (KFF 2023). While co-circulation of respiratory viruses has

been studied through clinical and wastewater surveillance, there remains a knowledge gap in understanding their co-occurrence and trends in indoor air.

In this study, we aim to (1) provide evidence for the airborne transmission of SARS-CoV-2 in the late- and post-pandemic era by examining indoor environments without known positive occupants; and (2) investigate the co-occurrence and potential seasonality of SARS-CoV-2, RSV and influenza A virus (IAV) in indoor air. We focus on university institutional spaces, which can be considered “semi-public” environments with controlled access and specific intended users such as students, faculty, and staff. A total of 54 HEPA filters from portable air purifiers within a university building were collected between September 2022 and October 2023. Viral RNA was extracted from particles accumulated on the filters and quantified for SARS-CoV-2, RSV, and IAV using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Findings from this study will enhance our understanding of the co-occurrence and seasonality of multiple respiratory viruses in shared indoor spaces and support the development of strategies for long-term airborne infection control.

Methods and Materials

Sample collection

Our sampling locations are situated within the Fielding School of Public Health at the University of California, Los Angeles (UCLA), specifically in the Center for the Health Sciences (CHS) Building, which is a seven-story structure that includes offices, conference and community spaces, and research laboratories. To protect building occupants during the pandemic, the UCLA Fielding School deployed Blueair HEPA purifiers in all non-laboratory rooms. For this study, we collected HEPA filters from purifiers installed in eight classrooms, five conference rooms, and one community room (Figure 1). Different Blueair models (211+ Auto, 311 Auto, and 411) were deployed, with the number and type of units determined by room size and each model’s clean air delivery rate (CADR). Room areas ranged from 311 to 1,542 ft², with larger rooms equipped with more units of higher CADR. All sampled rooms were located within the same building and served by a central HVAC system with ceiling-mounted supply. The number of diffusers was generally proportional to room size, with each diffuser covering approximately 80–160 ft². Table S1 summarizes room areas, seating capacities, air exchange rates (AER) for classrooms with air purifiers operating, and purifier specifications.



Figure 1. Representative images of the three room types examined in this study.

CADR values were obtained from the Blueair product website.

In rooms with a single purifier, the unit was typically positioned against a wall near a central location to access a power socket; in rooms with multiple units, devices were placed along different walls to connect to separate outlets. The purifiers were intended to operate during school hours (approximately 9:00 AM to 5:00 PM, Monday–Friday). Classrooms were typically open around 8:00 AM, at which point purifiers were turned on, and closed around 6:00 PM, when purifiers were turned off. Although the exact operational schedule was not continuously monitored, the purifiers generally followed this routine. Occasional interruptions occurred when faculty or students temporarily turned off a unit, but overall, operation was consistent across rooms and representative of real-world use conditions in the building.

Sampling was conducted in three rounds to capture seasonal variations: first round (September 2022–March 2023, six months) represented fall-winter; second (March–June 2023, three months) represented spring; and third (June–October 2023, four months) represented summer. We acknowledge that fall and winter are distinct seasons; however, we use the term ‘seasons’ to refer to the three sampling periods for simplicity in terminology and discussion. At the beginning of each round, new filters were installed in air purifiers and collected at the end of the period. A total of 19 filters were collected for the first round, 18 for the second, and 17 for the third. From each filter, two to four pieces (approximately 7 cm × 29 cm) were cut for further processing, resulting in 161 samples (Table 1) for analysis. All filter samples were stored at –80 °C until further analysis.

RNA concentration and extraction

Each filter sample was eluted in 20 mL of 1× phosphate-buffered saline (PBS) by shaking at 3000 rpm for 15 min. The blank control (BC) was prepared by eluting new, unused filters under the same conditions. From each eluate, 10 mL was processed for

Table 1. Number of filter samples analyzed using qPCR, by season and room type.

Sampling period	Classroom	Conference	Community	Total
Fall-Winter, 2022–2023, 6 months	39	11	6	56
Spring, 2023, 3 months	38	9	6	53
Summer, 2023, 4 months	42	3	7	52
Total	119	23	19	161

viral RNA concentration and extraction, while an additional 10 mL of 1× PBS was processed as a negative process control (NPC). To ensure extraction quality, sample eluates, BC eluates, and NPC samples were all spiked with 0.025% (v/v) Bovine Coronavirus (Calf-Guard® Bovine Rota-Coronavirus Vaccine, Zoetis Inc., Kalamazoo, MI, USA) as a process control prior to extraction. Samples were concentrated using Nanotrap Microbiome A Particles (Ceres Nanosciences, Manassas, VA, USA), followed by RNA extraction using the NucleoMag DNA/RNA Water kit for water and air samples (Macherey-Nagel, Düren, Nordrhein-Westfalen, Germany), according to the manufacturer’s instructions. All extracted RNA samples were stored at –80 °C until further analysis.

RT-qPCR analysis

SARS-CoV-2

N1 primer/probe set was obtained from the 2019-nCoV RUO primer/probe kit (Integrated DNA Technologies (IDT), Coralville, IA, USA). A synthetic DNA gBlock standard (sequence: GACCCCAAATCAGCGAAATG CACCCCGCATTACGTTTGGTGGACCCCTCAGATTC AACTGGCAGTAACCAGA) from IDT was *in vitro* transcribed and purified for use as the gene standard. Thermocycling conditions used for amplification were: reverse transcription at 50 °C for 15 min, enzyme activation at 95 °C for 2 min, followed by 45 cycles of denaturation at 95 °C for 3 s and annealing/extension at 55 °C for 30 s.

RSV

Primer and probe oligos were purchased from IDT. The sequences and final concentrations were: forward

primer: GGCAAATATGGAAACATACGTGAA (0.5 μ M), reverse primer: TCTTTTCTAGGACA TTGTAYTGAACAG (0.25 μ M), and probe: FAM-CTGTGTATGTGGAGCCTTCGTGAAGCT-BHQ1 (0.5 μ M) (Fry et al. 2010). Quantitative genomic RNA from RSV from the American Type Culture Collection (ATCC; VR-955DQ, Manassas, VA, USA) was used as the standard. Thermocycling conditions were: reverse transcription at 50°C for 10 min, enzyme activation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 15 s and annealing/extension at 55°C for 1 min.

IAV

Primer and probe oligos were purchased from IDT. The sequences and final concentrations were: forward primer: GACCRATCCTGTCACCTCTGAC (0.8 μ M), reverse primer: AGGGCATTCTGGACAAATCGTCTA (0.8 μ M), and probe: FAM-TGCAGTCCTC GCTCACTGGGCACG-BHQ1 (0.2 μ M) (Shu et al. 2011). Quantitative genomic RNA from IAV from ATCC (VR-95DQ) was used as the standard. Thermocycling conditions were: reverse transcription at 50°C for 15 min, enzyme activation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 15 s and annealing/extension at 55°C for 30 s.

BCoV

Primer and probe oligos were purchased from IDT. The sequences and final concentrations were: forward primer: CTGGAAGTTGGTGGAGTT (0.9 μ M), reverse primer: ATTATCGGCCTAACATACATC (0.9 μ M), and probe: FAM- CCTTCATATCTATAC ACATCAAGTTGTT-IABkFQ (0.25 μ M) (Greenwald et al. 2021). A synthetic RNA Ultramer (sequence: TAGAACTGGAAGTTGGTGGAGTTTCAACCCAG-AAACAAACAACCTTGATGTGTATAGATATGAAG-GGAAGGATGTATGTTAGGCCGATAATTGAGG) from IDT was used as the standard. Thermocycling conditions matched those for SARS-CoV-2. Thermocycling conditions were identical to those used for SARS-CoV-2.

General protocol

All viruses were quantified using RT-qPCR with the TaqMan Fast Virus 1-Step Master Mix for qPCR

(Thermo Fisher Scientific, Waltham, MA, USA). Reactions were performed in duplicates on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) in total reaction volume of 20 μ L, including 5 μ L of RNA template. Nuclease-free water served as the no-template control (NTC) sample.

QA/QC

All BC, NPC, and NTC samples performed as expected, yielding negative results for the target genes of SARS-CoV-2, IAV, and RSV. All standard curves exhibited R^2 values greater than 0.99, with slopes ranging from -3.1 to -3.5 (a slope of -3.32 is considered ideal and indicates 100% amplification efficiency) and efficiencies between 90% and 110%. The limit of detection (LOD) for each target virus was reported as the lowest standard concentration, expressed in gene copies (gc) per μ L, that were consistently amplified across all qPCR assays. To provide additional context, we also report the average cycle threshold (Ct) values of the lowest standard concentrations and the maximum amplified Ct values, and the total number of plates and reactions analyzed for each viral assay (Table 2). All assays were conducted using 384-well plates. It is noteworthy that for BCoV, the Ct value of the lowest standard concentration (20 gc/ μ L) was approximately 32, suggesting that the true assay sensitivity may be higher than indicated by this conservative LOD estimate.

The BCoV recovery efficiency for each sample was calculated using relative recovery efficiency based on the formula below:

$$\text{Relative recovery efficiency} = 2^{-(Ct_{\text{sample}} - Ct_{\text{NPC}})} \times 100\%$$

where Ct_{sample} is the Ct value of the BCoV target in samples, and Ct_{NPC} is the Ct value in NPC samples spiked with BCoV. Samples with BCoV recovery efficiencies below 1% were excluded from further analysis (Kantor et al. 2022), resulting in 34 out of 161 samples (21%) being excluded and leaving 127 samples, among which the median BCoV recovery efficiency was 65% (interquartile range (IQR) = 44–109%).

Table 2. Limits of detection (LODs), corresponding Ct values, and assay performance metrics for each virus.

Virus	LOD, gc/ μ L	Ct of lowest standard (mean $\pm$ SD)	Maximum amplified Ct	Total plates (n)	Total reactions (n)
SARS-CoV-2 (N1)	1	35.5 $\pm$ 1.1	41.4	4	464
RSV	1	36.2 $\pm$ 0.6	38.0	2	400
IAV	1	35.3 $\pm$ 0.4	42.1	2	400
BCoV	20	32.2 $\pm$ 0.9	38.7	4	232

Statistics

RT-qPCR analysis yielded two types of outcomes: the positive rate (the proportion of samples in which viral RNA was detected) and the concentration of viral RNA in these positive samples (expressed as gene copies per filter sample).

To assess differences in positive rates, a Pearson's Chi-square test was used to evaluate the overall effect of room type and season. For pairwise comparisons between specific room types (e.g., classroom vs. conference room) or seasons (e.g., spring vs. summer), Fisher's exact test with Bonferroni Correction was applied.

To evaluate viral RNA concentrations, we first assessed normality using the Shapiro-Wilk test, which indicated that the data did not follow a normal distribution. Therefore, a non-parametric Kruskal-Wallis one-way ANOVA on ranks was used to determine whether significant differences existed across seasons or room types. When overall differences were found, Dunn's test with Bonferroni Correction was applied for pairwise comparisons.

All analyses were performed using R software (version 4.3.3), with statistical significance defined as $p < 0.05$ after adjustment.

Results and discussion

Positive rates of SARS-CoV-2 RNA on air purifier filter samples

SARS-CoV-2 RNA was detected in all three types of rooms in this study. As shown in Table 3, the overall detection rate for SARS-CoV-2 RNA across all samples was 21% (27/127). The filter sample numbers in the denominator differ from those in Table 1 because samples with a BCoV recovery efficiency below 1% were excluded. By season, detection rates were 27% (12/44) in fall-winter, 16% (5/31) in spring, and 19% (10/52) in summer. By room type, the detection rates were 15% (15/99) for classrooms, 43% (6/14) for conference rooms, and 43% (6/14) for the community room. Seasonal detection rates for each room type are provided in Table 3.

Previous studies have shown that portable air purifiers with HEPA filters effectively remove SARS-CoV-

2 from the air. One study reported that after installing portable air purifiers, 4 out of 5 households (80%) with COVID-19-positive occupants had no detectable SARS-CoV-2 RNA in air samples, while prior to installation of air purifiers, all air samples from these households tested positive for various SARS-CoV-2 genes (Rodríguez et al. 2021). Another study found that 7 out of 16 (44%) air samples in self-isolation rooms were positive for SARS-CoV-2 RNA during the sham period when HEPA filters were removed (Myers et al. 2022). Our findings further confirm that HEPA filters in portable air purifiers can capture airborne SARS-CoV-2 virions, with 21% of the filter samples tested positive. Notably, unlike previous studies that sampled healthcare settings or indoor spaces occupied by known COVID-19-positive individuals, our study focused on semipublic indoor spaces without known infectious individuals, either symptomatic or confirmed positive. This likely explains our lower positive rate compared to the above studies, while aligning more closely with a recent investigation in university classrooms in Toronto, Canada, conducted over a similar period (November 2022–February 2023), which reported a 12.2% positivity rate in vacuumed filter dust (Hoskin et al. 2024). In our study, from September 2022 to March 2023, the positivity rate for classroom filters was 19% (Table 3). UCLA follows the Los Angeles County Department of Public Health (LAC DPH) COVID-19 and Acute Respiratory Illness (ARI) Guidelines for Educational Settings, which require students who test positive, are symptomatic, or have a fever to isolate and refrain from participating in regular campus activities (Los Angeles County Department of Public Health (LAC DPH) 2025a; UCLA 2025). However, because there is often a window between infection and symptom onset, students may unknowingly come to campus while infectious. This explains our detection of SARS-CoV-2 RNA in the filters of portable air purifiers deployed in these semipublic, institutional spaces.

Pearson's Chi-squared test revealed a statistically significant difference in positive rates across different types of rooms ($p = 0.007$), suggesting that the likelihood of detecting SARS-CoV-2 RNA on air purifier filters varies by room type. This variation may relate to differences in room location and usage patterns.

Table 3. SARS-CoV-2 RNA positive rate, by season and room type.

Sampling period	Classroom	Conference	Community	Total
Fall-Winter, 2022–2023, 6 months	19% (6/31)	38% (3/8)	60% (3/5)	27% (12/44)
Spring, 2023, 3 months	19% (5/26)	0 (0/3)	0 (0/2)	16% (5/31)
Summer, 2023, 4 months	10% (4/42)	100% (3/3)	43% (3/7)	19% (10/52)
Total	15% (15/99)	43% (6/14)	43% (6/14)	21% (27/127)

For example, the community lounge, located on the first floor, serves as a common entry and gathering area and is likely frequented by a broader and more transient population throughout the day. In contrast, classrooms are located on the upper floors (3rd to 7th) and are primarily accessed by students attending scheduled classes, while conference rooms, located on the 1st floor and 7th floor, typically require reservations and have more restricted access. Although the overall test was significant, none of the individual pairwise comparisons using Fisher's exact test remained significant after applying the Bonferroni correction. This could be due to the small sample sizes for community and conference rooms. It may also suggest that the observed differences arise from a combination of factors, such as ventilation system operation, room size, and occupancy, that influence particle movement and HEPA filter capture efficiency. All these factors were inherently embedded within the classification of "room type," rather than being attributable to room type alone.

Pearson's Chi-square test and Fisher's exact test, applied to the count data, did not reveal a significant seasonal effect on the SARS-CoV-2 RNA positive rate in air purifier filter samples. However, differences in sampling duration should be considered when interpreting the observed trends, as longer sampling periods may include more shedding events, leading to a higher positive rate. To reduce this potential bias, we normalized the positive rates by the number of months in each sampling period, allowing fairer comparison across sampling periods of different lengths. Table 4 presents the sampling-duration-adjusted (month-normalized) positive rates for each sampling period by room type. The month-normalized positive rates appear more similar across sampling periods. It should be noted that this normalization does not represent the true monthly positive rate, which may vary within each sampling period, but rather serves to reduce bias arising from unequal sampling durations.

Both the raw positive rates and the month-normalized positive rates indicated no significant differences among the three sampling periods, in contrast to local clinical surveillance data from the LAC DPH (Los Angeles County Department of Public Health (LAC DPH) 2025b). Figure S1 displays the LAC DPH weekly surveillance data covering the same time frame as our

study. Two small peaks in clinical positive rates were evident: one is between week 47 of 2022 (late November) and week 2 of 2023 (mid-January), another one is between week 30 and week 39 of 2023 (late July to late September), respectively falling into the fall-winter and summer periods in our study.

Two reasons may explain the absence of similar peaks in our results. First, our multi-month filter sampling likely smoothed out short-term spikes: in spring, extended sampling increased the chance of detecting SARS-CoV-2 RNA from sporadic shedding events during generally low circulation, whereas in fall-winter and summer, extended sampling included many low-circulation weeks in addition to peak weeks, dampening apparent seasonal differences. Second, both peaks in the surveillance data coincided with holiday and vacation periods, when building occupancy was low. Reduced occupancy likely decreased airborne virus emissions, causing our filter samples to miss the short-term peaks seen in the clinical data.

Concentrations of SARS-CoV-2 RNA on air purifier filter samples

Among the 27 positive samples, SARS-CoV-2 RNA concentrations ranged from 23 to 471 gene copies per sample, with a median of 118 (IQR = 70). Figure 2 summarizes these concentrations by (a) room type

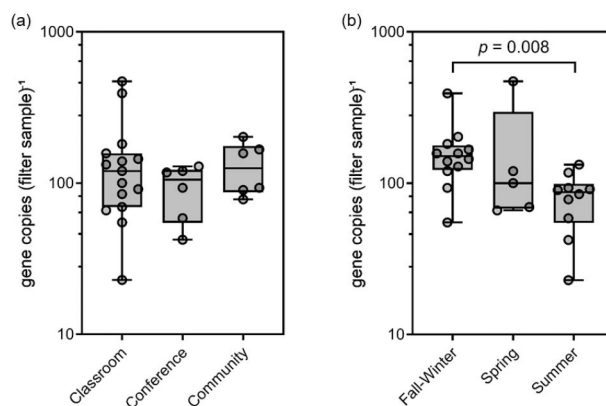


Figure 2. Box plots showing SARS-CoV-2 RNA levels (gene copies per filter sample) categorized by (a) room type and (b) season. Each circle represents an individual positive sample. The lines from top to bottom indicate the maximum, upper quartile, median, lower quartile, and minimum values. Statistically significant differences ($p < 0.05$) are indicated with corresponding p -values.

Table 4. Sampling-duration-adjusted (month-normalized) positive rates for different sampling periods.

Sampling period	Classroom	Conference	Community	Month-normalized positive rate
Fall-Winter, 2022–2023, 6 months	3.23%	6.25%	10%	4.55%
Spring, 2023, 3 months	6.41%	0	0	5.38%
Summer, 2023, 4 months	2.38%	25%	10.7%	4.81%

and (b) season. Median concentrations were similar across rooms: 120 gene copies (IQR = 74) in classrooms, 105 (IQR = 52) in conference rooms, and 125 (IQR = 74) in the community room. Seasonally, higher median levels were observed in fall-winter (151; IQR = 44), followed by spring (100; IQR = 51), and summer (87; IQR = 29).

A Kruskal-Wallis one-way ANOVA on ranks showed no statistically significant differences in SARS-CoV-2 RNA concentrations among the three room types. However, a significant difference was observed across seasons ($p=0.008$). Post hoc analysis using Dunn's test after Bonferroni correction revealed that RNA levels in fall-winter were significantly higher than those in summer ($p=0.006$). The statistical analysis of SARS-CoV-2 RNA concentrations revealed a different pattern from that of the positive rate. While the positive rate reflects the likelihood of detecting viral RNA on a filter sample, the concentration represents the amount of RNA present when detected. Room type was significantly associated with the positive rate but not with RNA concentration, whereas season had no significant effect on the positive rate but was significantly associated with RNA concentration.

This difference may be explained by the nature of our sampling method. As discussed before, because the air purifiers continuously collected airborne particles over a multi-month period, the extended sampling duration likely increased the probability of capturing RNA from sporadic viral shedding events, leading to relatively stable detection rates across seasons. In contrast, the total amount of RNA accumulated on the filters better reflects the overall viral load in the air during that sampling period, which varied significantly between fall-winter (6 months) and summer (4 months). Higher RNA concentration in fall-winter may be partly due to seasonal differences in ventilation practices. It was reported that in California, offices are often overcooled in summer, and HVAC systems typically deliver more outdoor air during this season (Bennett et al. 2012), leading to higher effective ventilation compared with winter. Reduced ventilation during fall-winter could lead to lower air exchange rates and longer indoor residence times for viral particles, while higher ventilation in summer can dilute viral aerosols more effectively, resulting in lower RNA concentrations in the air on the filters.

However, these differences may be confounded by the varying lengths of the sampling periods. Longer deployments can increase not only the likelihood of detecting a positive sample but also the total accumulation of RNA on the air filters. To account for this,

we normalized the total SARS-CoV-2 RNA gene copies detected on each filter by the number of months the filter was deployed, yielding an average monthly RNA load. As shown in Figure 3, after normalization, no significant differences were observed between any two sampling periods. It is important to note, however, that RNA load on filters reflects both cumulative deposition and RNA degradation over multi-month deployments. Therefore, normalization by sampling duration does not fully capture differences in airborne concentrations. This adjustment is intended only to provide an alternative interpretation of the results.

Assuming viral RNA was evenly distributed across the entire filter surface, we estimated total RNA loads by extrapolating from the concentration measured in the filter sample (which was a sub-filter cut from the full filter, see Methods and Materials) based on the ratio of its area to the total filter area. This yielded an estimated range of 3.66×10^3 to 7.54×10^4 gene copies per filter, with a median of 1.44×10^4 (IQR = 1.34×10^4) gene copies. This estimate is highly approximate, as only 21% of filter samples tested positive, indicating substantial spatial heterogeneity in viral RNA distribution, often with some pieces testing positive while others from the same filter did not. Using the estimated RNA concentration across the entire filter, along with the manufacturer-provided air purifier flow rate and a HEPA filter efficiency of 99.97%, we estimated the airborne concentration of SARS-CoV-2 RNA. The purifiers were intended to operate during school hours, approximately 8 h per day. To account for potential variability in operation, including occasional temporary shutdowns, we conservatively assumed a 50% duty cycle (4 h per day).

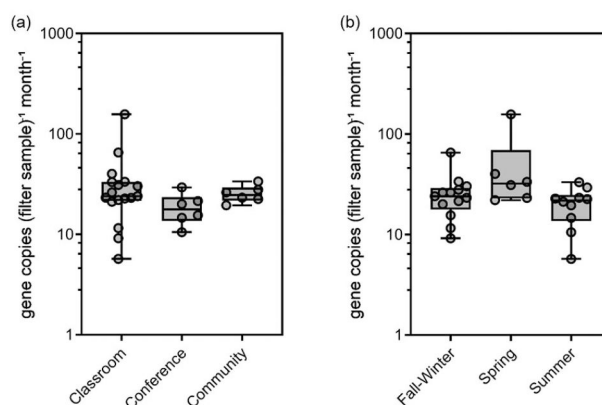


Figure 3. Box plots showing normalized SARS-CoV-2 RNA levels (gene copies per filter sample per month) categorized by (a) room type and (b) season. Each circle represents an individual positive sample. The lines from top to bottom indicate the maximum, upper quartile, median, lower quartile, and minimum values.

Under this conservative scenario, estimated airborne RNA concentrations reached up to 0.52 gene copies/ m^3 , representing an upper-bound estimate. Longer actual runtimes would increase the processed air volume and further reduce the calculated concentrations. This low concentration reinforces the notion that even at reduced levels, SARS-CoV-2 remains airborne and detectable.

Detection of RSV and IAV RNA on air purifier filters

The positive rates and viral RNA concentrations for RSV and IAV virus were substantially lower than those observed for SARS-CoV-2. RSV RNA was detected in only 2 out of 127 filter samples (2%) and IAV RNA was detected in 5 out of 127 filter samples (4%), all at very low concentrations. Table S2 summarizes the room type, sample details, and detection information for all RSV- and IAV-positive filter samples.

Several factors may explain the low detection rates and concentrations of RSV and IAV in our indoor air samples: First, the number of cases or shedding events for RSV and IAV may have been much lower compared to SARS-CoV-2 during our study period. According to national surveillance data, RSV case numbers were nearly two orders of magnitude lower than those for COVID-19 and influenza (Luo et al. 2023). Another important factor is the proportion of asymptomatic infections, which varies across viruses and influences the likelihood that infected individuals enter shared indoor spaces. Meta-analyses have estimated that the asymptomatic proportion of SARS-CoV-2 infections (especially during the Omicron period) can be as high as 26–32% (Wang et al. 2022; Yu et al. 2022), whereas estimates for influenza range from 8% to 11% (Ip et al. 2017; Biddle et al. 2025). This suggests that individuals infected with SARS-CoV-2 were more likely to be asymptomatic and unknowingly present in public or shared settings, thereby shedding virus into the air. In contrast, individuals infected with IAV were more likely to experience symptoms and stay home, resulting in fewer opportunities for airborne transmission in indoor public spaces.

Second, viral shedding rates differ by virus. SARS-CoV-2-infected individuals may emit higher viral loads than those infected with RSV or IAV. While comparisons are complicated by variations in sampling methods and reporting units, studies have detected SARS-CoV-2 RNA concentrations in exhaled

breath up to 10^7 gene copies per liter (Malik et al. 2021; Johnson et al. 2022), whereas IAV RNA concentrations have been reported up to 10^3 gene copies per minute (equals to 167 gene copies per liter given that an adult exhales about 6 liters of air per minute) (Fabian et al. 2008; Yan et al. 2018; Pleil et al. 2021; Shetty et al. 2024; Tay et al. 2025). Based on these numbers, the viral RNA burden in exhaled breath from SARS-CoV-2 cases may be substantially greater than that of IAV.

Third, viral RNA stability and degradation may differ between pathogens, influencing detection. A recent systematic review compiled decay rate data for SARS-CoV-2, IAV, and RSV RNA in aerosols and on surfaces from 35 studies and found that RSV and IAV have higher median decay rates than SARS-CoV-2 (Bandara et al. 2023), indicating faster degradation and possibly reduced detectability on filters.

Taken together, these differences in infection prevalence, asymptomatic proportions, viral shedding rates, and environmental stability may help explain the much lower detection of RSV and IAV in our filter samples. However, more standardized and comparative studies are needed to confirm these patterns across different respiratory viruses. Overall, due to the small number of positive indoor air samples for RSV and IAV in our study, we are unable to draw firm conclusions about their seasonality in indoor environments, which contrasts with the trends observed in clinical and wastewater surveillance data. For example, clinical surveillance data confirm that RSV and influenza activity peaked in early winter (around November 2022) across both LAC as well as the U.S. (Los Angeles County Department of Public Health (LAC DPH) 2025b; Luo et al. 2023), and wastewater surveillance data from northern California support similar seasonal trends (Boehm et al. 2023).

Implications and limitations

This study provides evidence that SARS-CoV-2 RNA can be detected in indoor air through HEPA purifier filters, even in settings without known symptomatic or confirmed positive individuals, reinforcing the role of airborne transmission under real-world conditions. Notably, SARS-CoV-2 was detected across all sampling periods with substantially higher positivity rates and RNA concentrations than RSV and IAV. Statistical analyses suggested potential associations between room types and sampling periods in relation to SARS-CoV-2 presence: room type was associated with the positive rate, while season was associated

with viral RNA concentration. These findings highlight the importance of combining detection rates and quantitative viral levels to more fully characterize airborne transmission risks.

Several limitations should be noted. First, there are limitations in sample collection. The extended sampling duration (4–6 months) likely resulted in RNA degradation on the filter, which could have led to an underestimation of both positive rates and viral concentrations. Extended sampling periods may also smooth out short-term variations, potentially masking true seasonal differences. Additionally, the sampling durations were not uniform across all filters, meaning that results cannot be directly compared between sampling periods. Second, there are limitations in analytical methods. RT-qPCR detects viral RNA but cannot determine whether the detected material represents infectious virus. Therefore, our results indicate the presence of viral genetic material rather than viable viruses. Furthermore, because our analysis relied on cutoff filter samples rather than the entire filter, the uneven distribution of RNA across the filter surface could have led to under- or overestimations of both positive rates and viral loads. Third, the interpretation of associations in our study should be made with caution. While our analyses point to possible links between room type or season and SARS-CoV-2 detection patterns, the underlying factors remain uncertain. Factors such as actual occupancy and ventilation characteristics likely influence viral presence and purifier performance. Future studies that integrate these contextual data will be important to clarify the mechanisms behind the observed patterns.

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