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# Occupational exposure monitoring of airborne respiratory viruses in outpatient medical clinics

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**ABSTRACT**

Exposure to airborne respiratory viruses can be a health hazard in occupational settings. In this study, air sampling was conducted from January to March 2023 in two outpatient medical clinics—one primary care clinic and one clinic dedicated to the diagnosis and treatment of respiratory illnesses—for the purpose of assessing airborne respiratory virus presence. Work involved the operation of a BioSpot-VIVAS$^{TM}$ as a stationary air sampler and deployment of NIOSH BC-251 bioaerosol samplers as either stationary devices or personal air samplers worn by staff members. Results were correlated with deidentified clinical data from patient testing. Samples from seven days were analyzed for SARS-CoV-2, influenza A H1N1 and H3N2 viruses, and influenza B Victoria- and Yamagata-lineage viruses, with an overall 17.5% (17/97) positivity rate. Airborne viruses predominated in particles of aerodynamic diameters from 1–4 $\mu$m and were recovered in similar quantities from both clinics. BC-251 samplers (17.4%, 15/86) and VIVAS (18.2%, 2/11) collected detectable viruses at similar rates, but more numerous BC-251 samplers provided greater insight into virus presence across clinical spaces and job categories. 60% of samples from reception areas contained detectable virus, and exposure to significantly more virus ($p = 0.0028$) occurred at reception desks as compared to the "mobile" job categories of medical providers and nurses. Overall, this study provides valuable insights into the impacts of hazard mitigation controls tailored to reducing respiratory virus exposure and highlights the need for continued diligence toward exposure risk mitigation in outpatient medical clinics.

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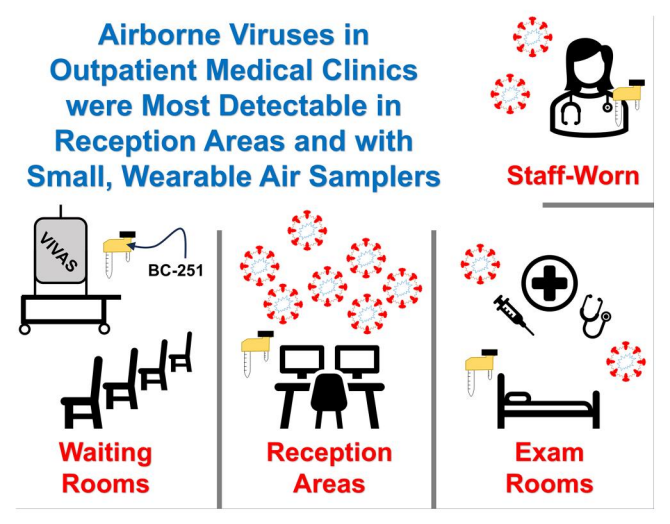
**GRAPHICAL ABSTRACT**




## 1. Introduction

Occupational risk assessment and mitigation encompasses a vast program in the United States focused on protecting worker health and safety that is enshrined in public law (Occupational Safety and Health Act 1970). The Occupational Safety and Health Administration (OSHA) within the U.S. Department of Labor publishes an array of standards (OSHA 2023) focused on reducing workplace injuries and health conditions from exposure to toxic substances. The National Institute for Occupational Safety and Health (NIOSH), which is part of the Centers for Disease Control and Prevention (CDC), supports OSHA by conducting research and managing a Health Hazard Evaluation program (NIOSH 2019) designed to build knowledge about workplace health hazards and advise employers on how to mitigate them. This combined effort is intended to help lessen the human suffering associated with workplace illness and injury linked to an estimated $58 billion (Liberty Mutual 2023) in annual monetary costs to employers from medical fees and lost-wage payments. Stewart et al. (2003) argued for a more complete accounting of workplace illnesses and injuries by assessing lost productivity, including both health conditions and reduced work performance that amounted to $225.8 billion annually, which would cost $383.3 billion in 2024 after adjusting for inflation. Workplace hazard mitigation is vital to the sustainment of both human health and economic efficiency.

Workplace safety regulations are less established for infectious diseases beyond bloodborne pathogen (BBP) programs (OSHA 2023), though a proposed "Infectious Diseases" rule could establish regulatory guidelines to protect against respiratory pathogens (Department of Labor 2023). The historical focus on BBP likely stems from the severe diseases associated with BBP transmission through injuries like hypodermic needle punctures. Still, Karve et al. (2013) reported an employer cost of $623,248 per 100,000 plan members related to influenza during the H1N1 pandemic season of 2008-2009. Suwantarat and Apisarnthanarak (2015) reported that healthcare personnel are at particular risk of infection by pathogens causing emerging viral diseases like severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Ebola virus disease, and avian influenza acquired in patient-care settings. de Perio, Kobayashi, and Wortham (2020) likewise highlighted the impact of occupationally-acquired respiratory viral infections from influenza viruses and SARS coronavirus 2 (SARS-CoV-2). Though chronic BBP like human immunodeficiency virus (HIV) may warrant special attention due to the elevated cumulative health costs experienced by people infected with them (Cohen et al. 2020), the typically acute illnesses stemming from respiratory virus infections still constitute occupational hazards, especially for healthcare workers encountering novel viruses.

The COVID-19 pandemic spurred many employers in the healthcare sector and beyond toward workplace modifications for the mitigation of SARS-CoV-2 exposure risks (Godderis et al. 2023; Misra, Ponnam, and Banerjee 2023; Ingram et al. 2021). Those modifications proceeded within a still-extant circumstance where universal approaches to biological risk

evaluation are lacking, and where many occupations have no formal quantitative microbial risk assessments from which to gauge risk determinations (Burzoni et al. 2020). Studies discussing bioaerosol exposures do exist for some sectors like healthcare (Pena et al. 2021; Coelho and García Díez 2015) and waste management (Schlosser 2018), where pathogen presence is expected, but such assessments usually do not delineate exposure risks by job category at the same worksite. Also, understanding how specific workplace modifications, like improved indoor air change rates, may mitigate exposure risks for specific airborne pathogens and thereby prevent disease propagation, remains a knowledge gap within the aerosol science profession (Wang et al. 2021). To improve understanding about airborne/"through the air" (World Health Organization 2024) occupational exposure risks to viruses, characterizations of pathogens present in occupational environments are necessary (Santarpia et al. 2023), and the incorporation of job-differentiated measurements linked with ambient air quality metrics like relative humidity (RH), temperature, and carbon dioxide ($CO_2$) concentration may help improve associated risk assessments. The cataloging of robust knowledge gained from such characterizations and the application of lessons learned from the pandemic to the design of indoor environments can help improve public health (Taylor et al. 2023).

Accurate measurement of airborne viruses can be complicated by many factors. Environmental metrics like RH (Aganovic et al. 2021; Dabisch et al. 2021), temperature (Dabisch et al. 2021; Riddell et al. 2020; Cutler et al. 2012), sunlight (Dabisch et al. 2021; Schuit et al. 2020; Daikoku et al. 2015), and air exchange rate (Thornton et al. 2022; Li et al. 2007) all affect the recovery of viruses (Santarpia et al. 2024). Virus concentrations in air are typically low (Mainelis 2020), and only the infectious (viable) fraction is of concern when considering health risks since inactive viruses cannot cause disease. Airborne particle collection mechanisms used within common air samplers can inactivate viruses, as can dehydration during sampling and processes required to recover viruses from collection media (Pan, Lednicky, and Wu 2019). Air samplers can be large, noisy, and hampered by power supply limitations which can consign them to areas less desirable for virus collection (Wilson et al. 2022), and those samplers that have shown promise toward the conservation of viable viruses often have those limiting characteristics (Banholzer, Jent, et al. 2024; Wilson et al. 2022). Therefore, studies attempting the collection of viable viruses may ultimately yield underestimations of exposure risk simply because desirable samplers could not be used where exposures most likely occur. Consequently, occupational exposure studies often use personal air samplers that go with workers but may be less ideal for viable virus conservation. The existence of this tradeoff between maximizing the conservation of viable viruses and collecting samples at points of exposure calls for investigation into how well stationary area samplers can accurately characterize occupational exposures to respiratory viruses.

Healthcare facilities often have access to clinical testing data that can serve as an additional tool to evaluate pathogen exposure risks. If tracked temporally, those results can provide insight into pathogen prevalence within a facility. In fact, studies involving the collection of pathogens from ambient air sometimes incorporate the collection of samples from both individuals and the environment (Alsved et al. 2023; Wong et al. 2023; Cheng et al. 2020) because that provides valuable information about the presence of pathogen emission sources. However, clinical results alone only give generalized information about exposures to which staff might be subjected. Correlations between pathogen quantities detected in clinical samples and emitted to the environment can vary widely by pathogen and by the characteristics or activities of infected individuals (Lai et al. 2023; Tsang, Wong, and Mui 2023; Morawska et al. 2022; Yang et al. 2021; Buonanno, Stabile, and Morawska 2020). Clinical data alone may be insufficient to explain occupational exposure risks without data from the environment. Further, it is known that pathogen presence in the environment can differ by pertinent exposure pathways (Jones 2021) and work areas (Birgand et al. 2020) within medical clinics, so facility-level data tracking may miss spatial or individual variations in exposure. Still, clinical testing results are easily accessible; so, comparisons between them and air sampling data may reveal important details that help explain pathogen exposure risks within healthcare facilities.

This study involved the collection of air samples from two health clinics, where one operated mainly in a pre-pandemic configuration and a second operated with pandemic-specific risk mitigation measures in place. It was designed to achieve four objectives. First, data related to influenza A and B viruses and SARS-CoV-2 were compared by clinic, allowing this work to offer unique insight into the effects of COVID-19 pandemic exposure risk mitigations. Second, air sampling was done using a large, stationary water condensation-based air sampler and smaller personal samplers either worn by staff or operated as stationary

samplers. Outcomes therefore offer insights into the comparative benefit of using different sampler types for occupational exposure monitoring in similar settings. Third, air samplers were associated with staff members by job type to allow comparisons related to work-specific exposures. Finally, the study involved the collection of clinical testing data compiled concurrently with air sampling data, thereby allowing investigation into whether air sampling can suitably serve as a reliable surrogate for the use of patient testing data to determine staff exposure risks.

## 2. Materials and methods

This study was approved by the University of Florida Institutional Review Board (IRB) under protocol number IRB201903396.

### 2.1. Sampling site

Air sampling occurred in an outpatient facility at a collegiate healthcare center. Samples were collected from two clinics located in different sections of the same building. Both clinics were on the ground floor of a multi-story building, and both clinics were located along exterior walls with at least one doorway directly accessing outside. One clinic was operated in its designed configuration as a primary care clinic, herein labeled as the "Blue" clinic. The other clinic was dedicated to respiratory illness treatment, herein labeled as the "Red" clinic, and modified with engineering and administrative controls intended to reduce risks associated with respiratory virus exposure.

Waiting areas in both clinics typically had only a couple of patients in them at any given time, and the area of the Red clinic was especially empty as patients were quickly moved into exam rooms. Receptionist work areas in both clinics were separated from the waiting room by a service window that was kept shut when not interacting with patients (Figure S1). However, the receptionist work area in the Blue clinic was fully sectioned off from exam rooms, whereas in the Red clinic it was open to a hallway leading to exam rooms. Three-to-four patient exam rooms typically were used in the Red clinic and six in Blue. Red clinic appointments were 15-min but were variable in the Blue clinic due to the wider range of services offered. Administrative protective measures in the Red clinic beyond appointment intervals included an emphasized need for mask-wearing by patients and staff members and the enhanced ventilation of patient exam rooms following appointments by the activation of window-mounted exhaust fans. Further, time spent by staff in exam rooms with patients was limited, and administrative work not completed concurrently during exams was completed in separate staff office spaces.

The clinics were serviced by separate heating, ventilation, and air conditioning (HVAC) systems. Airflow measurements were taken using an airflow capture hood (model 420, Testo SE & Co. KGaA, Germany) at each clean air supply and room air return in each exam room, waiting area, and reception area sampling point. In the Red clinic, measurements were also taken at the exam room windows with and without the window-mounted exhaust fan operating. Accounting for airflow into and out of the sampling areas, the air exchange rate in air changes per hour (ACH) was calculated by,

$$ACH = \frac{\max\left(|inflows|, |outflows|\right)}{room\ \ volume} \times 60 \frac{\min}{hr} \qquad (1)$$

where *inflows* and *outflows* indicate volumetric airflow rates ($m^3$/min) and *room volume* represents the size of the sampling area ($m^3$). The maximum value among inflows and outflows was used because this was taken to represent the most accurate accounting of new air moving into the space. Airflows at the Red exam room windows were inflows with the window fan off and became outflows when the fan was switched on. Room volumes were determined using a laser measure (GLM165-40, Robert Bosch GmbH, Gerlingen, Germany).

RH, temperature, and $CO_2$ concentrations were recorded using a portable indoor air quality meter (model CO250, Exterior Technologies Inc, USA). Measurements were taken before and after each sampling at each sampling point, and an average of the two were used to define the conditions applicable to each sample.

### 2.2. Air samplers

Two types of air samplers were used for this study. First, a water condensation-based sampler, BioSpot-VIVAS™ (VIVAS, Model 300-P, Aerosol Devices Inc, USA), was used as a stationary air sampler. The operational principles used by this device for the collection of particulates from ambient air have been described in detail previously (Eiguren Fernandez, Lewis, and Hering 2014; Hering, Spielman, and Lewis 2014; Hering and Stolzenburg 2005), and the device has been used successfully both during laboratory work (Nannu Shankar et al. 2024) and field sampling (Vass et al. 2022, 2023; Nannu Shankar et al. 2022; Lednicky, Lauzard, et al. 2020; Lednicky, Shankar, et al. 2020) to conserve the

viability of respiratory viruses collected from the air. A brief explanation of VIVAS operation is included in the online supplementary information (SI). Due to the size of VIVAS and the sound levels produced by it during operation, this sampler was kept exclusively within the waiting areas (Figure S1).

The second sampler type was the National Institute for Occupational Safety and Health (NIOSH) Bioaerosol Sampler (Model BC-251), which likewise has been described previously (Cao et al. 2011; Lindsley, Schmechel, and Chen 2006). A brief description of the sampler is included in the SI. Samples were processed as reported in Vass et al. (2022) using the same VTM used in VIVAS samples. Seven BC-251 samplers and pumps were used.

At stationary sampling points, BC-251 samplers were positioned with their inlets facing the most likely location of patient-staff interactions. Samplers generally were located in the expected breathing zones of staff (1-2 m high) except for reception samplers, which were placed at the breathing zone height for seated staff members. The samplers were located within 1 m of the receptionist or the patient-staff interaction area that they were intended to characterize so they could most accurately represent staff exposures. The sampler in the Blue exam room differed since it had to be farther from the interaction area than was desirable due to space availability (Figure S2). For samplers worn by staff members, a BC-251 was attached to the shoulder strap of the backpack within the wearer's breathing zone with the sampler inlet facing away from the front of the wearer (Figure S3).

### 2.3. Sampling protocol

Samples were collected from 11 January to 3 March, 2023, on Wednesdays and Fridays. That date range coincided with the typical peak cold and flu season in northcentral Florida. Sampling days were selected according to practical considerations, like the availability of the sampling team, and clinic throughput considerations, accounting for reliably low appointment fill rates on Mondays. It was known that patient loading regularly differed between morning and afternoon clinic hours. Therefore, this study attempted to distinguish between those periods by conducting three-hour samplings in the morning and afternoon of each sampling day. Samples were collected from 9:00 AM to 12:00 PM on Wednesdays and 8:00–11:00 AM on Fridays due to differences in clinic staff meeting schedules. Afternoon samples were collected from 12:30–3:30 PM on both days.

Only one VIVAS was available for sampling, so the collection of samples from waiting areas was done alternatively in Blue and Red clinics. The same was true of reception area samples. In those cases, samplers were moved between clinics during the break between morning and afternoon samplings. On the next sampling day, the morning and afternoon locations were swapped to ensure that both times of day were characterized in both clinics. Likewise, the weekday on which the samplers were present in each clinic in the morning and afternoon was alternated each week. Enough BC-251 samplers were on-hand to allow consistent placement in exam rooms for both morning and afternoon collections on each sampling day.

Reception area samplers were used to characterize exposures by administrative staff working at those locations, but staff that moved around the clinics wore BC-251 samplers in backpacks. Staff from four job types wore samplers: providers (physicians, physician assistants, and advanced practice registered nurses), scribes, nurses (registered nurses), and medical assistants. Patient interaction times were comparable among nurses and medical assistants. Scribes always accompanied providers while conducting their tasks. Therefore, only two groupings were used to describe the "mobile" samplers: (1) providers, composed of providers and scribes, and (2) nurses, composed of nurses and medical assistants. Samplers and backpacks were issued to staff volunteers at the start of each sampling iteration, and the appropriate clinic and job category were recorded.

### 2.4. Sample analysis

Samples were analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) for the genomic RNAs of SARS-CoV-2 and Influenza A H1N1 and H3N2 viruses and by conventional RT-PCR followed by gel electrophoresis for Influenza B Yamagata- and Victoria-lineage viruses. Primer and probe sets used for SARS-CoV-2 were the Centers for Disease Control and Prevention (CDC) N1 and N2 assays (Lu et al. 2020). Sets for influenza viruses were designed based on the most recent WHO protocols for the molecular detection of influenza viruses (World Health Organization 2021). Only a subset of collected samples, selected for analysis by referencing clinical test data and identifying days with the highest virus presence, was analyzed due to constraints on time and analytical resources. Information regarding RT-PCR primers, probes, and cycling conditions is presented in Table S1.

Air sampling data were paired by day with de-identified clinical testing data. All patients reporting to the Red clinic received a test for SARS-CoV-2 and some also received a test for influenza A/B viruses. Clinical samples were analyzed using a Sofia® 2 Immunoassay Analyzer (model 20299, QuidelOrtho Corporation, CA, USA).

Culturing of viable viruses was planned for samples which held virus detectable by RT-qPCR at quantification cycle ($C_q$) values $\leq$37 because that limit included the largest $C_q$ for air samples containing both PCR-detectable virus and viable virus in past work done by this research group (Vass et al. 2022, 2023). Sample $C_q$ values were too high to warrant culturing; therefore, only results from molecular tests are reported in this study.

Additional details related to sample analysis are included in the SI.

### 2.5. Data analysis

Data were tabulated in Microsoft Excel and then imported into R for statistical analyses and figure production. Data were apportioned differently depending on the comparisons being made. Since BC-251 samplers divide collected particles into three size-defined stages, results from the separate stages of the same sampler were combined when making assessments that required no consideration of particle size. When it was important to know particle sizes attributable to collected virus, BC-251 stage samples were kept separate. Virus concentrations were analyzed and reported as genomic equivalents per liter of air (GE/L) after normalization by Equation (2) to account for the variable particle diameters collected by the stage of the air sampler,

$$Normalized\ Concentration = \frac{Concentration}{\Delta \log_{10} d_{p,50}} = \frac{Concentration}{\left(\log_{10} \frac{Upper\ \ d_{p,50}}{Lower\ \ d_{p,50}}\right)} \quad (2)$$

where *Concentration* is the value determined by molecular analyses, *Upper* $d_{p,50}$ is the particle size that corresponds to the point at which the previous stage is 50% efficient, and *Lower* $d_{p,50}$ is the smallest particle size collected by the current stage with 50% efficiency. For VIVAS and BC-251 Stage 3, the lower bound was defined as 0.01 $\mu$m. That boundary condition was selected to include the size of target viruses (Laue et al. 2021; Bouvier and Palese 2008; Stanley 1944) and allow for the detection of virus RNA that was not fully packaged and potentially present in particles smaller than fully packaged virions. The upper bound for VIVAS and BC-251 Stage 1 was defined as 10 $\mu$m, which is a size that is characteristic of inhalable particles that can affect the thoracic region of the human respiratory system (Baron 2017).

In both forms of data set apportionment, results within analyzed categories failed normality assumptions due to small sample size, zero-inflation stemming from a large number of samples in which a detectable amount of virus was not recovered, or distortions from various factors impacting the dataset, such as time of day. Therefore, non-parametric statistical tests were used to evaluate the dataset with the level of significance held at $\alpha = 0.05$. Holm corrections were applied to p-values for the reduction of false-positives. The median was used as the measure of centrality, and spread was described with the interquartile range (IQR).

Logistic regression was applied to explain the effect of $CO_2$, RH, and temperature on the detection of viruses. To facilitate interpretation, data were binned into two groups for each environmental variable. Temperature was divided at 24.4 °C (OSHA 2022), RH at 30% (OSHA 2011), and $CO_2$ at 1000 ppm (ASHRAE 2022) based on indoor air quality recommendations from governmental or advisory bodies generally pertaining to human comfort in the workspace. The binary detection or non-detection of virus was set as the response variable and the environmental measurements as predictor variables in a fixed effects model. Values <24.4 °C, >30% RH, and <1000 ppm $CO_2$ were considered normal values and so were held as reference conditions for each variable. Therefore, the model evaluated the effect of the addition of an extreme environmental condition on the likelihood of virus detection.

Additional details related to data analysis are included in the SI.

## 3. Results

### 3.1. Sampling site conditions

Room volumes and airflow rates are shown in Table 1. Air changes per hour (ACH) in the Red clinic (median = 7.14) was higher than the Blue clinic (median = 2.15).

The measured ACH values in the two clinics were clearly separated (Figure 1a), and a one-tailed Wilcoxon rank sum test supported the graphical interpretation that ACH was significantly lower in the Blue clinic ($p = 0.05$).

Data related to temperature, RH, and $CO_2$ concentrations are listed in Table 2 and depicted in Figure S4. Kruskal-Wallis rank sum tests indicated that at least

**Table 1.** Air exchange rates measured at sampling locations.

| Clinic | Room | Room Volume [$m^3$] | Supply [$m^3$/min] | Return [$m^3$/min] | With Window Fan Off [$m^3$/min] | ACH | Median ACH |
|---|---|---|---|---|---|---|---|
| Blue | Exam | 30.8 | 1.10 | −0.93 | | 2.15 | 2.15 |
| | Reception | 40.3 | 0.91 | −0.85 | | 1.35 | |
| | Waiting | 35.7 | 1.33 | −1.36 | | 2.28 | |
| Red | Exam | 19.7 | 0.93 | −0.85 | 1.81 | 8.36 | 7.14 |
| | Reception | 11.9 | 1.08 | −1.42 | | 7.14 | |
| | Waiting | 37.1 | 3.48 | −3.94 | | 6.36 | |

Airflow rates were recorded during a single visit to the clinic during normal operating hours. ACH was calculated by Equation (1). Positive values denote airflows into the room and negative values indicate airflow out of the room. When the window fan in the red exam area was tuned on at the highest setting, the airflow became -20.30 $m^3$/min and ACH in that space became 64.4.

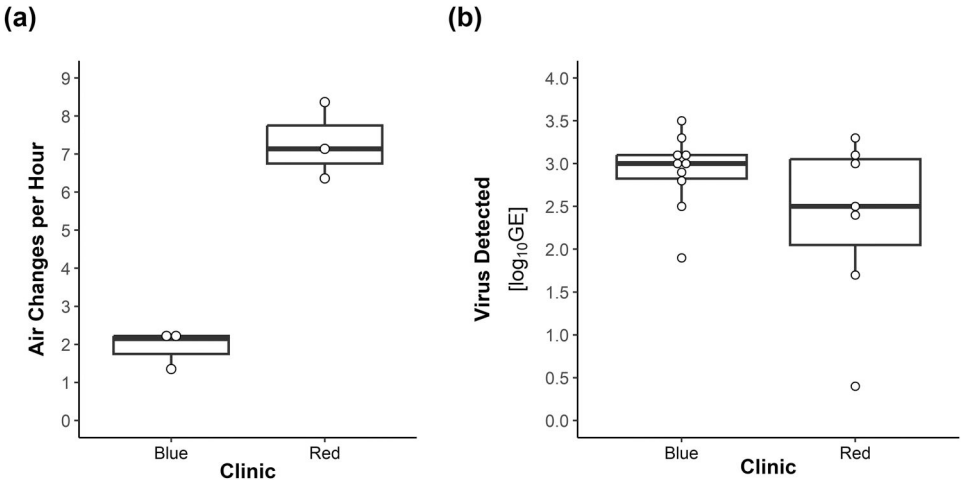



**Figure 1.** (a) Air change rates, and (b) virus recovery from air samples, shown by clinic. GE refers to Genomic Equivalents of virus detected.

**Table 2.** Summary of environmental conditions during sampling.

| Clinic | Room Type | Temperature [°C] | | Relative Humidity [%] | | Carbon Dioxide [ppm] | |
|---|---|---|---|---|---|---|---|
| | | *Median* | *IQR* | *Median* | *IQR* | *Median* | *IQR* |
| Blue | Exam | 22.8 | 21.3–23.4 | 44.2 | 38.4–46.5 | 697.0 | 638.8–803.2 |
| | Reception | 24.2 | 24.0–24.2 | 40.8 | 36.8–43.2 | 813.0 | 759.5–871.5 |
| | Waiting | 23.0 | 22.3–23.6 | 43.6 | 38.8–44.7 | 698.8 | 652.1–817.5 |
| Red | Exam | 21.1 | 20.3–22.0 | 55.1 | 49.8–56.5 | 781.0 | 683.0–963.8 |
| | Reception | 22.6 | 22.0–23.1 | 54.8 | 51.5–55.8 | 825.5 | 758.5–855.0 |
| | Waiting | 21.9 | 21.0–23.0 | 54.9 | 45.0–55.5 | 789.0 | 772.0–1003 |

The "Red" clinic was designated as a respiratory illness clinic, and "Blue" was a primary care clinic. IQR ranges represent the Middle 50% of the associated non-parametric distributions.

one significant difference existed for RH ($p = 0.0011$) and temperature ($p = 0.0074$) between the Red and Blue clinics. No statistically significant difference existed for $CO_2$ ($p = 0.15$). Pairwise Wilcoxon rank sum tests indicated many significantly different temperature and RH conditions by sampling site. However, the application of Holm adjustments revealed that the sampling sites were mostly similar, as significant relationships were reduced to only one for RH between the Red and Blue exam rooms ($p = 0.021$).

ACH was positively correlated with RH ($p = 2.97E-08$) and negatively correlated with temperature ($p = 3.64E-05$) by Spearman's rank correlations (Table S2), but no correlation existed for $CO_2$ ($p = 0.12$) and detectable virus concentrations ($p = 0.56$). Virus concentrations were positively correlated with temperature ($p = 0.0060$) but not with RH ($p = 0.72$). $CO_2$ had a marginally significant correlation with detectable virus concentration ($p = 0.060$). All samples with detectable virus were collected when $CO_2$ was 669-1004 ppm, and the middle 50% of $CO_2$ concentrations for those samples was 743–829 ppm.

When evaluated by logistic regression to assess the impact of environmental conditions exceeding current guidance on virus detectability, only the presence of the more extreme temperature condition (≥24.4 °C) significantly increased the likelihood of detecting virus ($p = 0.0298$). All four samples collected when the

temperature was ≥24.4 °C were collected from the Blue clinic on 25 January, and 75% (3/4) contained detectable virus. A sensitivity analysis (Table S3) showed that the significance of the temperature effect was sustained for temperatures above the third quartile (23.1 °C, $p = 0.0391$) but was lost at the median (22.2 °C, $p = 0.0596$).

### 3.2. Clinical tests

A median of 24 (IQR = 15–30) patient-unique SARS-CoV-2 tests were administered in the Red clinic each day on days when air sampling occurred. The trend in our data set diverged from the municipal COVID-19 case rate and hospital admissions trends (Figure 2). Nearly all patients seen at the clinics were members of a collegiate student body. Our data reflect the effect of that group returning to the local area from winter break *en masse* around 9 January. We attribute the increase in clinical positives at our sampling site around 20 January to virus transmission within that student body during the first two weeks after returning to campus. Whereas seven-day average case rates across the municipality remained constant, case rates on campus spiked for roughly two weeks and then stabilized toward a trend largely aligned with the municipality. The median positivity rate for SARS-CoV-2 was 18.8% (IQR = 16.7–35.5%). Not all patients received flu tests, but a median of 16 (IQR = 15–23) were administered to unique patients each day with a positivity rate of 18.5% (IQR = 13.1–27.9%).

### 3.3. Air samples

Air samples were collected over 15 sampling days (no sampling occurred on Wednesday, 18 January, due to staffing constraints). Samples from five days were selected for analysis based on clinical tests (Figure 2), and two additional days (11 and 13 January) were also included because laboratory analysis began sequentially before clinical data were available for air sample prioritization. Analyses focused on days which seemed to have the highest potential for airborne virus recovery based on clinical data. 302 samples were analyzed by RT-qPCR and PCR followed by gel electrophoresis, of which 53 were negative controls collected in the clinics. Among the 249 field samples, 139 came from stationary samplers located in the exam, reception, or waiting areas, and 110 came from mobile samplers worn by staff members. 238 samples were collected with BC-251 samplers, and 11 with VIVAS. 20 of the 238 samples were not analyzed because they could not be located at the analytical lab. In instances where particle size fractionation was not pertinent, the three stages of each BC-251 sampler were combined into a single composite sample. For such comparisons, the number of BC-251 samples was 86.

Among the 86 BC-251 and 11 VIVAS samples, 17 (17.5%) contained virus detectable by RT-qPCR. No detectable virus was recovered from negative controls. Counts of samples with detectable virus are shown by sampling date in Figure 2. The detectability of virus in air samples was significantly correlated with the

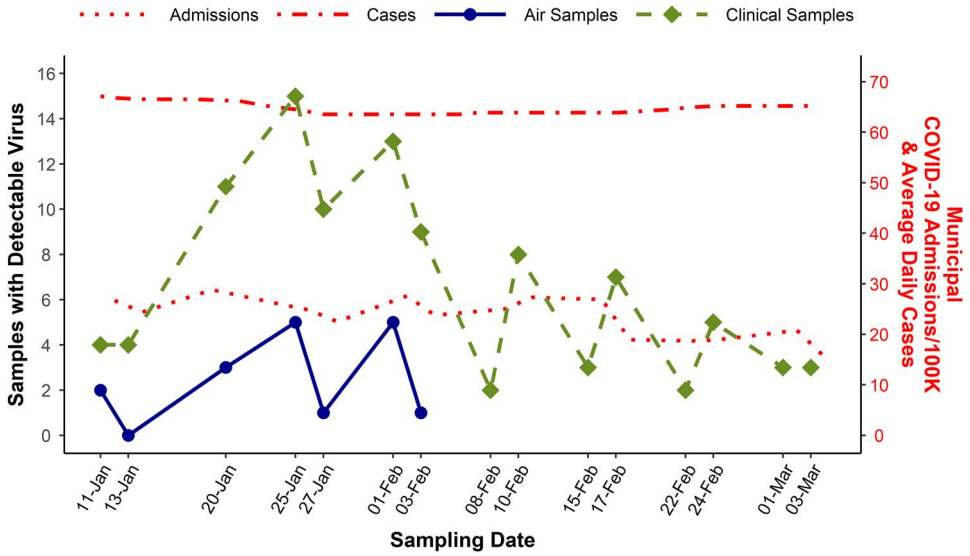



**Figure 2.** Samples with detectable virus by sampling date. Dashed lines with diamond points signify clinical tests and solid lines with circular points represent air samples. Data for municipal hospital admissions and daily COVID-19 case counts as 7-day moving averages are included for context and described by the right axis.

detectability of virus in clinical samples by Spearman's rank correlation ($p = 0.022$).

The positivity rate in the Red clinic was 15.6% (7/45) and in the Blue clinic was 19.2% (10/52) (Figure 3). Detectable virus quantities were similar between the clinics by a Wilcoxon rank sum test ($p = 0.56$, Figure 1b), and the sample positivity rates were likewise not statistically different by a chi-square test for independence ($p = 0.84$, Figure 3).

Samples were most frequently positive at stationary samplers located in reception areas (60%), followed by waiting areas (16%) and exam rooms (9.1%). 12.5% of mobile samplers collected detectable viruses. By chi-square tests for independence, the positivity rate among reception area samples was statistically different from both mobile ($p = 0.029$) and exam room ($p = 0.041$) samples, and the difference was only marginally insignificant between reception and waiting area samples ($p = 0.11$). Waiting area and mobile samples had higher positivity rates in the Blue clinic, and exam room samples had a higher positivity rate in the Red clinic. Reception areas in the two clinics had the same positivity rate. Differences were apparent graphically (Figure 3) between reception area rates and rates from samples categorized as either "Mobile" from the Red clinic or "Exam" from the Blue clinic. However, sample size reductions due to grouping by both clinic and sample category meant that no significant differences remained after p-value adjustments.

Samplings in the afternoons had a positivity rate of 20.4% (10/49), higher than the mornings (14.6%, 7/48), though the difference is not statistically meaningful ($p = 0.59$ based on Fisher's exact test). Airborne virus concentrations by time of day were not statistically different by a Wilcoxon rank sum test ($p = 0.44$), but the data showed contrasting groupings (Figure 4a). Viruses were collected more often and were detected at higher concentrations (GE/L) in the afternoons in the Blue clinic, whereas the opposite was true in the Red clinic. The positivity rate with the BC-251 (17.4%, 15/86) was nearly equal to VIVAS (18.2%, 2/11), but BC-251 samplers collected virus in more instances than did VIVAS (Figure 4b), thereby improving the depth of information available for this analysis. The greater collection with the BC-251 versus VIVAS is apparent graphically, though numerically the virus concentrations detected with both sampler types were not statistically different by a Wilcoxon rank sum test ($p = 0.93$).

Virus concentrations by instrument type remained similar when the dataset was reduced to only samples from the waiting areas ($p = 0.83$), where both instruments operated. VIVAS only recovered detectable virus from the Blue clinic waiting area ($n = 2$), whereas BC-251 samplers collected detectable virus from both the Blue ($n = 1$) and Red ($n = 1$) clinic waiting areas (Figure 5a). When considering only samples from the Blue clinic waiting area where both instrument types collected detectable virus, concentrations remained statistically similar ($p = 0.63$). Concentrations detected with the two devices were not similar, however, when comparing VIVAS samples from the waiting areas to the BC-251 samples from reception areas. The samples from reception areas indicated significantly higher

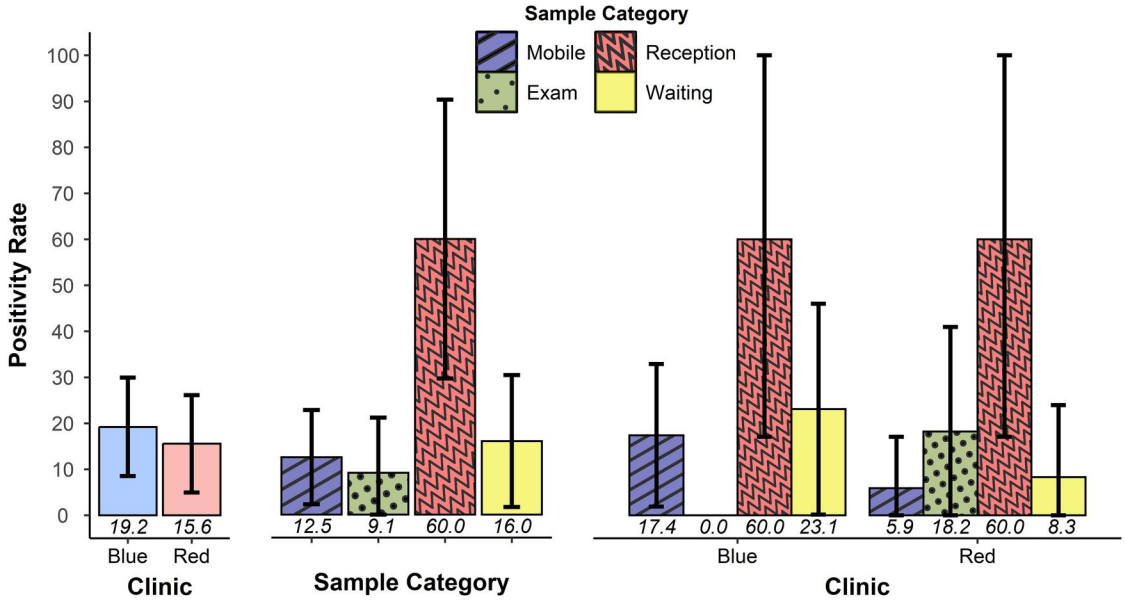



**Figure 3.** Positivity rates for virus detection in air samples displayed by clinic, sample category, and sample category within each clinic. Rates are listed in italics at the base of each bar. Error bars represent 95% confidence intervals, which widen as the data are divided into more groups and sample sizes decrease.

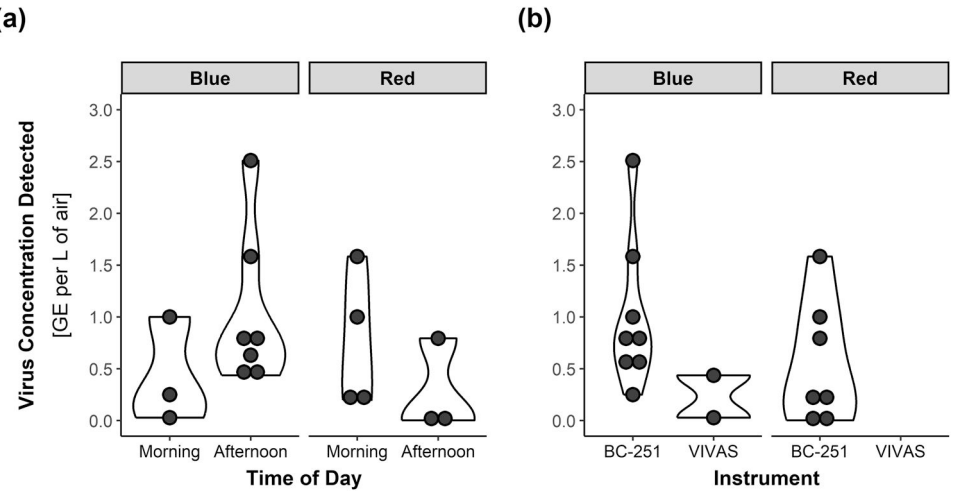



**Figure 4.** Virus concentrations in air are depicted by (a) time of day and (b) instrument used to collect the air samples. GE refers to Genomic Equivalents of virus detected. Both plots are sub-divided by clinic. Note that while only samples with positive values are depicted for ease of viewing, most samples did not contain detectable virus. Both detectable and non-detectable sample results were included in statistical tests.

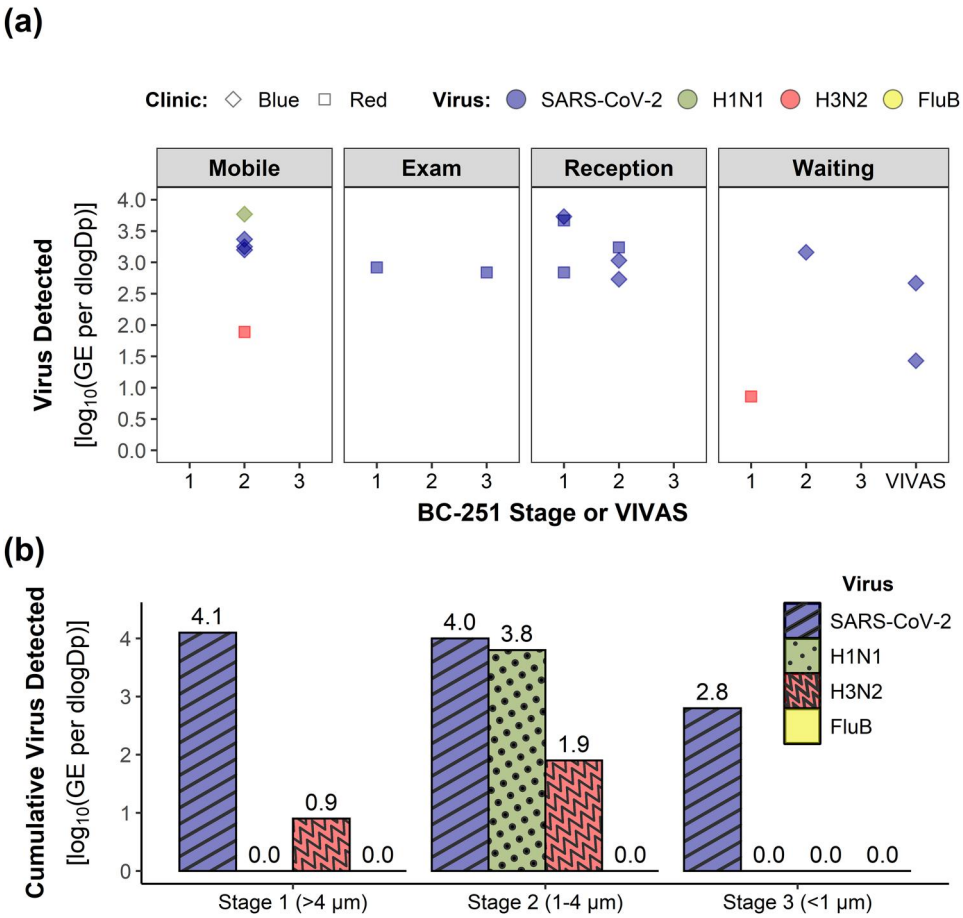



**Figure 5.** Detectable virus depicted by particle size. GE refers to Genomic Equivalents of virus detected. Particle cut sizes used to define bin sizes for normalization were as described in sections 2.2 and 2.5. (a) Concentrations of viruses in individual samples. The shape of the icons signifies the clinic in which the sample was collected, and the color denotes the virus detected. VIVAS was only operated in the waiting areas. Samples without detectable virus are not shown. (b) Cumulative amount of each virus—indicated by color—detected in each stage of the BC-251. Because VIVAS does not size-fractionate by particle diameter, the two VIVAS samples with detectable virus were excluded.

virus concentrations than were detected in VIVAS samples ($p = 0.033$).

Most samples with detectable virus (9/17) came from stage 2 of the BC-251 samplers (1–4 $\mu$m particles). The only sample positive for H1N1 was from stage 2, as was one of two samples with detectable H3N2, which contained 91.5% of all H3N2 collected (Figure 5). Influenza B virus was not detected in any samples. SARS-CoV-2 was more evenly distributed between stages than influenza virus A, with 50.8% in stage 1, 46.1% in stage 2, and 3.1% in stage 3. All samples with detectable virus from staff-worn devices were from stage 2 as were 50% (3/6) of such samples from reception area stationary samplers. Stage 1 (>4 $\mu$m particles) collected the second-greatest number of samples with detectable virus (5/17). Virus was detected in only one sample from stage 3 (<1 $\mu$m particles). Overall, when quantities of all detected viruses were combined by stage, 57.3% was collected in stage 2, 40.3% in stage 1, and 2.4% in stage 3.

The quantities of viruses detected in samples were different by job category as determined by a Kruskal-Wallis rank sum test ($p = 0.0068$, Figure 6).

Subsequent pairwise Wilcoxon rank sum tests with Holm p-value adjustments indicated that significantly more viruses ($\log_{10}$GE) were collected from reception areas than from samplers worn by medical providers ($p = 0.012$). The unadjusted result from comparing reception areas and samplers worn by nurses likewise showed significant difference ($p = 0.027$), but the significance became marginal after adjustment ($p = 0.054$) due to the smaller nurse-group sample size. Nurse and provider samples were not statistically different ($p = 0.46$).

The scope of work for all worn-sampler job categories were similar in that they involved intermittent close-proximity interaction with patients in exam rooms, so they were further combined into a single "mobile" category. When combined and compared with a Wilcoxon rank sum test, virus detected at the reception sampling areas was significantly different ($p = 0.00197$) than what was collected with staff-worn samplers. Figure 6 shows the greater amount of virus detected in reception area samples.

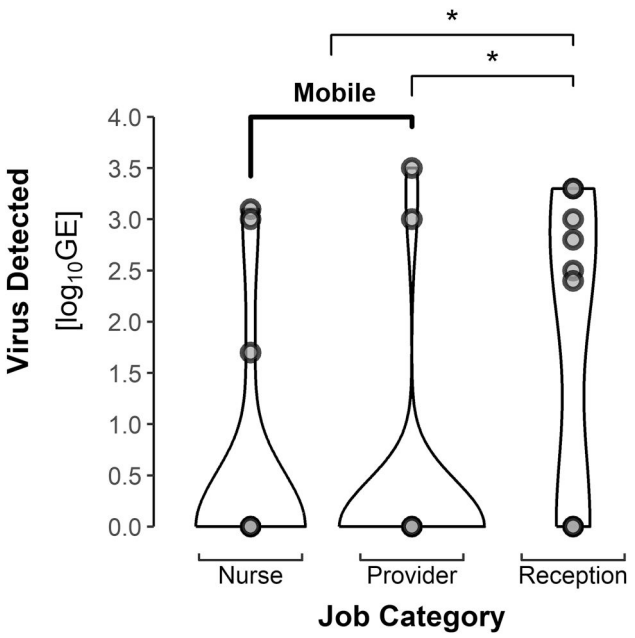



**Figure 6.** Virus detected by job category as recovered from samples collected with BC-251 samplers. GE refers to Genomic Equivalents of virus detected. Results show the low comparative counts of samples with detectable virus for jobs where samplers were worn, as indicated by the "Mobile" label, and jobs where staff were stationary (Reception). Significantly different relationships (adjusted p-value $\leq$ 0.05) are shown with an asterisk. Samples with non-detectable virus are shown as zero values, though the limit of detection is a higher value. Such an arrangement affects only the height of the violin plots, not associated statistical tests.

## 4. Discussion

### 4.1. Clinic comparisons

Linkage between increased cleaning or exchanging of indoor air and the reduction of airborne pathogen transmission has been well established. Past research has investigated *Mycobacterium tuberculosis* (Banholzer, Schmutz, et al. 2024; Riley et al. 1962; Wells 1955), measles virus (Riley, Murphy, and Riley 1978), *Streptococcus pneumoniae* (Hoge et al. 1994), SARS-CoV (Li et al. 2005), influenza virus (de Perio, Kobayashi, and Wortham 2020), and SARS-CoV-2 (Banholzer, Schmutz, et al. 2024; Zhang et al. 2024). Work by Riley, Murphy, and Riley (1978) based on work from Wells (1955) yielded the widely-used Wells-Riley model for the estimation of infection risk in indoor settings (Sze To and Chao 2010). That same model has been often modified to account for particular variables of interest, such as RH (Aganovic et al. 2021). Li et al. (2007) provided a helpful review of work relating air change rates and disease transmission before the COVID-19 pandemic, and Thornton et al. (2022) offered an updated review focused on HVAC design features. Both discussed the link between air exchange rate and disease transmission in greater depth.

That accepted correlation reinforced the expectation that the clinic with higher ACH would have reduced virus detection, which was bolstered by

additional protective measures in the Red clinic. Administrative controls like the limitation of appointments to 15 min resulted in reduced patient-staff interaction times and fewer patients sitting in the waiting area. Mask-wearing by patients was recommended and much more common in the Red clinic, though it was not expressly required. Window fan installation and operation between appointments constituted an engineering control intended to remove pathogens from exam room air. The use of personal protective equipment like N95 respirators provided staff protection in both clinics. Increased air changes, shorter and fewer appointments, and more common mask-wearing should have lowered virus detectability in the Red clinic, but detectability was similar between clinics. A confounding circumstance was the dedication of the Red clinic to respiratory illness diagnosis and treatment. Symptom-based patient-sorting likely biased the dataset, placing more virus emission sources in the Red clinic, thereby increasing virus detectability. Given that circumstance, similar virus detectability between clinics may be considered a testament to the importance of maintaining higher air changes in clinical spaces. In a primary care clinic with an ACH below the 4.0 recommended for general purpose outpatient exam rooms (ASHRAE 2020), virus quantities detectable in air samples were similar to those recovered from samples collected in a dedicated respiratory illness clinic.

The collection of detectable respiratory viruses in clinical spaces where patients with respiratory illnesses were not expected suggests the existence of an important consideration for staff exposures: unknown virus emission sources. Asymptomatic and presymptomatic exhalation of SARS-CoV-2 has been the subject of discussion in many studies in detail (Jefferson et al. 2022). Other studies have also reported high rates of asymptomatic influenza virus infections (Leung et al. 2015; Carrat et al. 2008). It is plausible that viruses detected in the Blue clinic came from such patients. It is also plausible that symptomatic patients ignored the clinic guidance because they needed services (non-respiratory) provided only in the primary care clinic. Alternatively, it could have been that staff members were themselves virus emission sources. While the potential for any combination of these circumstances taking place during air sampling makes it impossible to say from whom viruses originated, none negate implications related to exposure to respiratory viruses. Protections against respiratory viruses remain relevant for outpatient medical clinics because viruses can be present in the air, even when patients with respiratory illness symptoms are diverted elsewhere.

Whereas results from the Blue clinic indicate a need for enduring protective measures and improved respiratory virus exposure monitoring, results from the Red clinic highlight a success. In addition to the engineering controls implemented to improve ACH already discussed, the Red clinic administrative controls should be noted. 15-min appointment blocks implemented in the Red clinic helped ensure that patients could be moved quickly out of the waiting area. That reduced the virus recovery rate from the waiting area relative to the exam room (Figure 3). Similarly, since staff would limit their interactions with patients, entering the exam room to do assessments and then leaving to complete administrative requirements, and different staff would perform different tasks pertinent to their jobs, a given staff member wearing a mobile sampler spent only a fraction of the appointment time with a patient, thereby reducing exposure time. Deliberate workflow management may help explain why the positivity rate from mobile samplers in the Red clinic was lower than that of the stationary exam room samplers (Figure 3).

Conversely, in the Blue clinic, it was observed that the exam room allocated for sampling often was occupied last during clinic operational flow and frequently was empty during periodic sampler checks. In the waiting area, patients would be present in greater numbers than in the Red clinic. Procedures for patient movement in the Blue clinic likewise help explain the lack of virus recovery from the sampled exam room and comparatively higher recovery from the waiting area. Less-common patient mask-wearing in the Blue clinic likely also contributed to the higher mobile sampler positivity rate compared to the Red clinic. Taken together, positivity rates by clinic and sample category support the notion that by implementing a hierarchy of controls, respiratory virus hazards may be relegated to areas where they can be more effectively controlled, and staff can be protected from exposures. Results also suggest that in the absence of such controls, staff may be exposed more often to such health threats.

### 4.2. Job categories

One area where engineering and administrative controls appear to have not mitigated virus exposure risks was at reception desks. Samples from those areas had higher positivity rates than any other sample category in both clinics (Figure 3). Further, positive samples

collected at reception work areas outnumbered those from other job categories (Figure 6). The reception area samplers were positioned near the breathing zone of seated staff members. That meant that samplers were below the breathing zone of standing patients. As a result, the reception samplers could have been collecting more large particles present in patient exhalation plumes during speaking and coughing (Morawska et al. 2022; Johnson et al. 2011) than were collected by samplers in other categories positioned higher. This was borne out in the data, which showed that 50% of the samples with detectable virus from the reception area were from BC-251 stage 1 (>4 $\mu$m particles) and 50% from stage 2 (1–4 $\mu$m particles) (Figure 5). It would seem likely that the wearing of masks by patients would mitigate the spread of larger particles (Milton et al. 2013), but it has been shown that improper fit of masks can lead to a drastic reduction in their protective performance as aerosols escape from the poorly fitted sides (Brooks et al. 2021; Kolewe et al. 2020). Further, the use of fabric masks often fails to prevent the emissions of aerosols (Lindsley, Blachere, Law, et al. 2021; Lindsley, Blachere, Beezhold, et al. 2021), and some patients wore such face coverings. It likewise would seem like service windows between receptionists and patients should have reduced virus presence around staff members (Al-Rikabi et al. 2023; Bartels et al. 2022), something not borne out in these data. This possibly was due to openings in the service windows negating some benefit of the barriers (Dhanak et al. 2022), or it might serve as another indicator of staff as virus sources. Regardless, results in this study suggest that even with improved protective measures in patient care areas, staff regularly interacting with patients in non-clinical capacities may remain at increased risk of exposure to respiratory viruses in similar clinics. Clinics might consider mitigation measures like virtual appointments or remote patient registration and check-in to reduce interactions.

### 4.3. Particle sizes

Notwithstanding results at the reception areas, the greatest quantity of virus was collected in particles with 1–4 $\mu$m aerodynamic diameters (Figure 5), a size range that has been highlighted for its importance because it is possible for particles of such sizes to deposit in the lower respiratory tract (Morawska et al. 2022; Wang et al. 2021), and viruses reaching those deposition points can cause infections deep in the lungs. Our detection of more virus in the 1–4 $\mu$m particle size range corresponds well to an exhaled breath study (Milton et al. 2013), where influenza virus copies detectable in particles $\leq$5 $\mu$m were 8.8 times higher than what was detectable in particles >5 $\mu$m. Likewise, Yan et al. (2018) reported the collection of nearly twice as many samples positive for influenza virus in fine aerosols (0.05-5 $\mu$m) as in coarse (5–100 $\mu$m). Lindsley et al. (2010) detected only 23% of influenza RNA in 1–4 $\mu$m particles using BC-251 samplers, but another 42% was collected in particles <1 $\mu$m, which is likewise pertinent to infections in the lower respiratory tract. Also, it should be noted that particle size cutoffs are identified by the point at which a sampler is 50% efficient, so aerosols that are near 1 $\mu$m may deposit the viruses they carry into either collection stage. Our results showing the nearly equitable division of SARS-CoV-2 between the first two BC-251 stages agrees well with other published work. For instance, Alsved et al. (2023) reported the collection of most SARS-CoV-2 RNA in 0.94-2.8 $\mu$m particles and 90% in aerosols <4.5 $\mu$m using an eight-stage impactor. Chia et al. (2020) recovered SARS-CoV-2 from hospital patient rooms only in the first two stages of BC-251 samplers. However, it is important to remember that larger particles have the physical potential to carry substantially more viruses as their volumes increase. Our data simultaneously indicate that virus was most often detected in 1–4 $\mu$m particles, thereby signifying the presence of inhalation hazards in the sampling areas, while also showing how larger particles can deliver SARS-CoV-2 so that that virus detected in just two samples can be more abundant than what was collected in many more samples capturing smaller aerosols (Figure 5).

### 4.4. Stationary vs. mobile samplers

Past studies have demonstrated the collection and conservation of viable virus in air samples when using VIVAS in a variety of environments, including residences (Vass et al. 2022, 2023), an automobile (Lednicky et al. 2021), and even the same waiting area sampled in the Blue clinic during this study at the start of the pandemic (Lednicky, Shankar, et al. 2020). In this study, however, VIVAS did not do so. Though virus in VIVAS samples was collected at lower concentrations than most positive BC-251 samples (Figure 4), it failed to collect detectable virus in the Red clinic and only yielded two positive samples overall. Compared to co-located waiting area BC-251 samplers, VIVAS performed comparably. This demonstrated that the placement of samplers in less-ideal areas can

counteract the well-established concept that higher airflow samplers often better enable the collection of sufficient airborne pathogens to overcome detection limits (Borges et al. 2021; Pan, Lednicky, and Wu 2019). Our results suggest that stationary samplers may not adequately characterize exposure risks in settings where people frequently move around.

Perhaps stationary systems could do so if they were placed simultaneously at multiple locations, as was done with the BC-251 samplers. However, practical considerations likely would prevent this for more complex systems like VIVAS. For instance, fielding enough systems to design an adequate sampling plan may be cost-prohibitive, resulting in a low number of total samples and poor characterization of larger spaces as was the case here. Device sound levels prevented VIVAS operation in patient care areas and other work areas, and such limitations may exist in other occupational settings. Power supply requirements often compel the placement of VIVAS away from the most desirable locations. The effect of these limitations can be best appreciated by focusing on reception desks, staff areas to which VIVAS was closest. In both clinics, reception desks were separated only by service windows from the waiting areas. At such proximity, VIVAS in the waiting areas could have provided adequate representation of exposures where receptionists worked. An equivalent characterization of exposures would have been encouraging since it would have indicated that a sampler better able to conserve viable virus could be used in similar settings while avoiding negative impacts on work conditions. The results did not support that notion. Higher virus concentrations in reception areas indicated that using only VIVAS to characterize reception area exposures would have yielded an underestimation.

The results do suggest that smaller, quieter samplers like the BC-251 could potentially better characterize similar indoor spaces as stationary samplers than could VIVAS. BC-251 devices detected virus at stationary sampling points throughout the clinics, showing that the accessibility limitation can be overcome with similar systems. Lower costs allowed the use of seven BC-251 systems and better spatial characterization of virus presence. However, we did not see equivalent positivity rates between stationary and personal samplers (Figure 3). The number of positive samples from areas not specifically aligned with staff (exam rooms and waiting areas) were much less numerous than staff-aligned categories (Figure 5). The data and mentioned limitations suggest that, while stationary samplers can have utility for occupational exposure monitoring, they will best inform health risk assessments if they are specifically aligned to relatively stationary staff jobs, such as the receptionists in this study. Otherwise, the data here indicate that a stationary sampler may not provide sufficient information for hazard exposure estimates. For example, Figure 3 shows that a given exam room might have a higher (Red) or lower (Blue) rate of exposure to respiratory viruses than is truly detectable with samplers worn by staff. What is most important in an occupational exposure assessment are staff exposures. While stationary samplers can helpfully characterize spaces, samplers worn by staff members will likely best describe staff exposures.

### 4.5. Clinical tests vs. air sampling

Clinical test results yield information well-correlated with air sampling results (Figure 2) and so appear to be a good indicator of virus presence in the air of this sampling environment based on this dataset. This may seem to say that air sampling is not necessary, since a simpler test with reduced time and costs associated with collection and analysis can describe virus presence in the air. To an extent, that appears to be supported by the data here, though we recommend a continued focus on collecting data related to viable viruses to corroborate or contradict this correlation as it pertains to viruses that can impart illnesses. However, relying on clinical testing alone may not yield information about where staff exposure risks are higher or for which jobs exposure risks may be elevated. For example, without air sampling, virus exposures at reception areas might have been thought less than exam rooms. Further, clinical testing does not confer information about potential transmission from one host to another and so requires SI to inform staff about potential respiratory virus exposures. We suggest that while monitoring clinical testing data may be sufficient for managing a hazard mitigation program, work sites with known exposure risks for respiratory viruses should consider a hazard evaluation involving air sampling so that specific areas of concern can be identified.

### 4.6. Molecular tests vs. virus isolation

Since virus enumeration was obtained only by PCR, it could be the case that detected viruses were not viable. The lowest Cq value measured during this study was 37.3, indicating that virus concentrations in samples were low. In past work reported by this research group

(Vass et al. 2022, 2023), the highest Cq value associated with an air sample from which virus was successfully cultured was 36.3. It was not surprising that small quantities of virus were recovered from air since it is known that virus concentrations in air are commonly low (Mainelis 2020), but virus detection difficulty was compounded by the necessitated use of low-flow air samplers, which have contributed to virus non-detection when used in studies targeting SARS-CoV-2 (Borges et al. 2021) and influenza viruses (Ahrenholz et al. 2011). Nonetheless, given the resultant PCR data, it was determined that the probability of recovering viable viruses from such high-Cq samples was low, so isolation in cell cultures was not attempted. SARS-CoV-2 can be shed for days and even weeks after the cessation of viable virus emission (Puhach, Meyer, and Eckerle 2022) but often is not viable 6–8 days after symptom onset (Boucau et al. 2022). Therefore, the lack of virus viability data highlights a complicating factor for occupational exposure assessments of respiratory viruses: if a sampling does not recover viable virus, little can be said definitively about health risks. This common difficulty in collecting airborne viruses when using small, low-flow air samplers calls for the development of air samplers both small enough to meet accessibility demands of occupational health hazard assessments and also optimized for collection and conservation of viable viruses. Alternatively, methods for estimating viable virus exposure risks should be developed through laboratory studies for application in instances where the ideal viable virus recovery cannot be accomplished.

### 4.7. Additional recommendations and limitations

Recovery of viruses from ambient air has been very dependent on the sampling environment, and low virus detectability rates in air samples like the 17.5% reported here are common. Borges et al. (2021) provided a review of 25 air sampling studies employing various samplers in which many failed to collect detectable virus, though some like Lednicky, Lauzard, et al. (2020), Santarpia et al. (2020), and Kitagawa et al. (2023) reported greater success at collecting detectable SARS-CoV-2 from the air in medical facilities. A NIOSH Health Hazard Evaluation detected influenza A virus (H1N1) in only 3% (3/96) of samples from two dental practices when operating BC-251 samplers for 4–5 h (Ahrenholz et al. 2011). Birgand et al. (2020) reported in their review of 24 hospital air sampling studies targeting SARS-CoV-2 that samples were positive for viral RNA in areas close to patients (17.4%, 82/471), other clinical areas (8.4%, 20/237), and dedicated staff areas (12.3%, 15/122). Rule et al. (2018) recovered influenza A virus from 42% (53/125) of SKC button samplers worn for 6 h by staff in an emergency room, as well as 43% (28/96) of stationary button samplers. However, those samplers were not size-fractionated like the BC-251 but collected all material onto a filter. Zhou et al. (2023) collected detectable virus in 25% (63/252) of air samples with the stationary cyclonic air sampler Coriolis Micro (Bertin Technologies, France) during a controlled study where patients were inoculated with wild type SARS-CoV-2 and then observed in negative pressure isolation rooms. However, that sampler operated at 300 L/min for a short sampling time (10 min), collecting 3000 L of air per sample. The BC-251 samplers in our study collected 630 L of air over 3 h, and the VIVAS collected 1440 L. The decision to operate for only 3 h was based on an intentional effort to differentiate between the mornings and afternoons, and it was not altogether imprudent since other work has offered concerns about virus destruction in BC-251 samplers if operated for over 5 h (Ahrenholz et al. 2011). Yet, a consequence of that decision was reduced likelihood of exceeding detection limits, increasing potential for underestimation of virus presence. Though BC-251 samplers sacrifice total air volume collected compared to high-flow samplers, that limitation can be somewhat mitigated by longer operation. Longer samplings for occupational exposure studies are favorable because they give insight into the entire day instead of short points in time. Considering Rule et al. (2018) and this study, we suggest that future studies seek to characterize full shifts or workdays to reduce the likelihood of under-characterizing hazards. Ideally, samplers would be selected or designed to operate at 6–16 LPM, a range that covers the "at-rest" and "normal activity" ventilation rates of a healthy human adult (Pleil et al. 2021). We argue, however, that the information gained from using a particle size-fractionating air sampler holds valuable value for understanding the potential transmission mechanisms for respiratory viruses that it is still prudent to use such devices in occupational exposure studies, even at risk of sacrificing some ability to detect virus presence.

Environmental conditions like RH, temperature, and $CO_2$ concentrations have been investigated widely as variables influencing virus presence and viability in the air (Thornton et al. 2022; Dabisch et al. 2021), and $CO_2$ has been proposed as a metric for estimating bioaerosol presence (Gangwar et al. 2024; Burridge et al. 2022) because it is exhaled by humans and so

may therefore positively correlate with airborne virus presence. However, others have argued that $CO_2$ is not a good metric for estimating the concentration of particulates in ambient air (Zhang and Bluyssen 2023), which suggests that $CO_2$ concentrations may not be descriptive of viral aerosol concentrations. We did not see a correlation between $CO_2$ and either virus presence ($p = 0.060$) or ACH ($p = 0.12$) (Table S2). While intriguingly close to the significance threshold, both correlations deceptively derive from poorly fitted models. The ACH correlation was affected by the clustering of air change rates by clinic and wide variation in $CO_2$ concentrations in both clinics (Figure S5). The $CO_2$ correlation with virus concentrations was simply aligned with the zero-inflated data (Figure S6), and detectable concentrations were not well predicted. A more robust dataset would be needed to draw conclusions about the relationship between airborne viruses and $CO_2$ concentrations, but our results do provide insight into the detectability of airborne viruses within the commonly issued guideline of 1000 ppm $CO_2$, a metric grounded primarily in the control of human body odor, not airborne pathogens (ASHRAE 2022). Monitoring $CO_2$ concentrations concurrently with the collection of airborne viruses from occupational settings in future studies could help establish a guideline for $CO_2$ grounded in the mitigation of airborne virus exposure risks.

Other environmental data presented here emphasize the importance of establishing such guidelines. It was apparent by logistic regression that temperatures exceeding 24.4 °C (76 °F) significantly increased the likelihood of detecting virus in air samples (Table S3). That effect remained significant even as the temperature was reduced to 23.1 °C (74 °F), though the low number of samples in the "high temperature" groups compels cautious interpretation of results from this dataset. The results do suggest that future studies should rigorously collect temperature and other environmental data for association with airborne virus detection. Such work could build a body of knowledge to aid the discernment of indoor air conditions most conducive to reducing airborne virus exposure risks.

This study involved several limitations not previously discussed. First, personal samples were not collected from staff members. Therefore, it remains unknown whether staff members were the emission sources of virus detected. This does not detract from the results because emitted virus presents an exposure risk to other staff members no matter the source, but future studies could be improved by the inclusion of staff sampling to better describe the circumstances of the study. Second, data points for airflow used to calculate ACH were collected on only one day because the equipment used was borrowed. Though we are confident that the data presented represent consistent conditions in the sampling environment because the HVAC system continuously operated, we nonetheless recommend that airflow be measured on each sampling day or at some greater frequency than was possible in this study to produce a more robust dataset. Despite these limitations, this study provides valuable information related to occupational exposure to airborne respiratory viruses in outpatient clinical settings.

## 5. Conclusions

Air within two outpatient medical clinics was sampled to characterize occupational exposure to airborne respiratory viruses. Results showed that both clinics had similar exposure risks despite one being dedicated to the diagnosis and treatment of patients exhibiting symptoms of illnesses caused by respiratory viruses. Data indicated that viruses were most often detected in 1–4 $\mu$m particles, signifying the presence of potential airborne/through-the-air virus transportation and inhalation hazards. Sample positivity rates showed that the implementation of a hierarchy of risk mitigation measures designed for respiratory viruses may reduce staff exposures. The results imply that in the absence of such controls, exposure to such health threats may remain even after the exclusion of symptomatic patients. Further, this work showed that even with improved protective measures in patient care areas, administrative staff regularly interacting with patients may be subject to increased exposure to airborne viruses. This study illustrated the importance of using personal air samplers in occupational hazard assessment plans and sampling for full shifts or workdays to avoid potential underestimations of risk from using only stationary samplers or shorter sampling times. A correlation existed between the number of samples with detectable virus in clinical tests and air sampling, but unique insights into spatially- and job-defined risks were possible only with air sampling. The authors recommend air sampling as part of occupational exposure monitoring for airborne respiratory viruses to better characterize risks within workplaces. They further encourage the development of methods for the estimation of exposures to viable airborne viruses in instances where the isolation of viable viruses cannot be accomplished.

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## Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability statement

The data that support the findings of this study are openly available Mendeley Data at DOI: 10.17632/5n282f2kxb.1.

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