Supplemental Materials

Title: Monitoring SARS-CoV-2 in Air and on Surfaces and Estimating Infection Risk in Buildings and Buses on a University Campus

Authors: Xin Zhang*, Jianfeng Wu*, Lauren M. Smith, Xin Li, Olivia Yancey, Alfred Franzblau, J. Timothy Dvonch, Chuanwu Xi, Richard L. Neitzel

Affiliations: University of Michigan School of Public Health, Department of Environmental Health Sciences. 1415 Washington Heights, Ann Arbor MI 48109, USA

Corresponding Author: Richard L. Neitzel (rneitzel@umich.edu); Chuanwu Xi, (cxi@umich.edu)

Corresponding Address: M6543 SPH II, 1415 Washington Heights, Ann Arbor MI 48109, USA

* These authors contributed equally to this work.

In this document, we provide summaries of our sampling locations, the corresponding U-M site specific mitigation strategies, and our field observation notes. We also describe the RNA extraction and processing protocols used in this study. In the third section, we provide the step-by-step calculation of infection probability using Quantitative Microbial Risk Assessment (QMRA) model.

Sampling Site Descriptions

In this study, we sampled classrooms, rehearsal rooms, office areas, cafeterias, buses, gyms, student activity buildings and Heating, Ventilation and Air-Conditioning (HVAC) system tunnels. The gym weight rooms are located on the ground floor with standard HVAC systems. Each piece of equipment was placed at least 6 feet apart, and each room allows 14 to 21 people in the room at the same time. The office building that we sampled is an open-space office area with about 50 cubicles. During the pandemic, less than 5 people were working on site each day. The college dining hall is another open space where students need to wait in line or eat at least 6-feet apart. About 240 people per hour passed by the dining reception stands. The classrooms and rehearsal rooms have the capacity of ~50 persons and COVID-19 policy reduced the capacity to 10 to 15 persons per room. The performance hall is the size of a small theater which could hold ~100 person in the audience but during the pandemic, each rehearsal only had up to ~12 people. The university buses are similar to city buses with 32-36 seats. Other places listed in Table S1, like the lobby in the teaching complex, the entrance and common area (lounge room), are open space with sparse traffic during the pandemic. Additionally, we positioned the sampling hose into the HVAC tunnel before recycled air entered the filtration panel, in order to see if virus was detectable in the recycled building air.

We recorded the environmental measurements before and after each sampling event. The average indoor temperature during the fall and winter semester was 22±1℃. On sampled university buses (with 50% windows open), the average temperatures were 22±3℃ in the fall and 18±3℃ during the winter semester, respectively. All sampling locations have standard Heating, Ventilation and Air-Conditioning (HVAC) systems, including university buses.

Mitigation Strategies

Over the course of the SARS-CoV-2 pandemic, U-M implemented a series of mitigation strategies based on the CDC guidelines. Building closures and work from home rules were put into place immediately after the declaration of the pandemic in March 2020. In the summer of 2020, the University began to allow certain buildings to be partially occupied and certain critical staff to come back to work. To allow for safe return, U-M implemented various University-wide and location-specific mitigation strategies. For example, the University tracked COVID-19 cases and testing results through campus surveillance programs. All campus buildings had controlled access and app-based screening requirements. All locations had enhanced cleaning and disinfection schedules and reduced occupancy, and masks and social distancing were required in all public spaces on campus, with one exception. The musical rehearsal rooms and performance stage did not require masks during wind instrument and vocal performances, and instead, required 15- to 30-minute breaks outside of the room between each rehearsal. Implementing screening and checking was less practical for bus operations, so campus buses increased fresh air flow by opening windows and installed barriers between the driver and passenger areas.

RNA Extraction and quantitative rRT-PCR

For each quantification, viral RNA was extracted using a TRIzol reagent method. We transferred 400 microliters (μL) of liquid from each surface swab or aerosol vial, respectively, into a 2-mL centrifuge tube and were inactivated using a 4:1 ratio of TRIzol LS Reagent (Invitrogen ThermoFisher Scientific, Waltham, MA, USA). The total RNA was then ready for RNA amplification. Total SARS-CoV-2 viral count was performed using quantitative real time reverse transcription-polymerase chain reaction (quantitative rRT-PCR) assay on an Eppendorf Mastercycler® RealPlex2 Real-Time PCR Detection System (Eppendorf, Hamburg, Germany). We used primer/probe sets from the US CDC that targets a region of the nucleocapsid (N1) gene (detailed information in Table S3). A synthetic DNA (VR-3276SD™, ATCC®, Manassas, VA, USA) was used as PCR positive control and to generate the standard curve. Each reaction contained 5 μL RNA template, 10 μl of 2× master mix (QuantiTect Probe RT-PCR kit, 204443, Qiagen, Inc., Hilden, Germany), 200 nM of primers and probes, and the reaction volume was adjusted to a final volume of 20 μL with RT-PCR grade water (delivered with the QuantiTect master mix). Thermal cycling reactions were carried out at 50°C for 30 minutes, followed by 95°C for 15 minutes and 45 cycles of 95°C for 10 seconds and 60°C for 30 seconds.

Standard Curve and Limit of Detection

All samples were run in duplicate. For genome copy quantification, a standard curve was generated with synthetic SARS-CoV-2 RNA. A series of 10-fold serial dilutions of the positive control, with concentration ranging from 10,000 to 1 copy per μL was used for each quantitative rRT-PCR plate. The quantitative rRT-PCR standard curves (Figure S1) have slopes that range

from -2.833 to -3.239. The curves' y-intercept range from 34.7 to 37.4. The PCR efficiency ranged from 93.5% to 107.2%. The R-square of standard curve is greater than 0.95.

We use previously published methods (Armbruster and Pry 2008; Bustin et al. 2009) to determine the limit of detection (LoD) for our quantitative rRT-PCR procedure. The assay LoD was initially calculated by running the standards of synthetic SARS-CoV-2 RNA in 6 replicates. The lowest concentration at which all the replicates were positive was considered as the LoD of the assay. In practice, each quantitative rRT-PCR plate had duplication of standards and the lowest concentration at which all the replicates were positive was considered as the LoD of the quantitative rRT-PCR plate.

For quality control, both the extraction blank and reagent blank were also included in each quantitative rRT-PCR plate to identify any carryover contamination and inhibition. Inhibition was detected by running the diluted RNA template along with the main sample to observe the effect on Cq value (Peccia et al. 2020).

Quantitative Microbial Risk Assessment (QMRA)

First, we converted the gene copy (gc) concentrations (gc/cm^2 for surface samples, or gc/L for air samples) into infective virus concentration (PFU/cm^2 or PFU/L) using $gc{:}\,inf$ (gc/PFU) ratio to estimate the number of viable viruses in the collected environmental samples, as shown in Equation 1:

$$C_{inf} = \frac{C_{gc}}{gc{:}inf} \qquad [1]$$

where C_{gc} is the genome copy concentration, C_{inf} is the infective virus concentration, and $gc{:}\,inf$ is a constant of 80 gc/PFU for SARS-CoV-2 (1).

We calculated the exposure dose through inhaled contaminated air in worst-case scenario (i.e., without face coverings) using an exposure model of aerosols generated from wastewater treatment processes (2). The first of our two exposure scenarios assumed a bus passenger taking a 5- to 15-minute ride and breathing regularly without a mask. The second scenario assumed an individual completing 30-50 minutes of moderate- to high- intensity interval training (MIIT to HIIT) and breathing vigorously without a mask in an indoor gym.

The inhaled dose d for a one-time stay was estimated using Equation [2]:

$$d_{air} = \frac{C_{inf}}{eff_{air}} \times IR \times t \qquad [2]$$

where eff_{air} is the recovery efficiency of the air samples, IR is the inhalation rate (L/min) and t is the duration of stay in minutes.

The surface contact exposure dose was calculated using a community surface contact model (3). This model assumes an exposure pathway in which the viable viral dose experiences two losses, first during a single hand-to-surface contact, and second during a single hand-to-mucus membrane contact.

The contacted dose for the contact exposure pathway was estimated using Equation [3]:

$$d_{surf} = \frac{C_{inf}}{eff_{surf}} \times TE_{sh} \times TE_{hm} \times FSA \qquad [3]$$

where eff_{surf} is the recovery efficiency of the sample swabs, TE_{sh} is the transfer efficiency of viruses between surfaces and hands, TE_{hm} is the transfer efficiency between hands and mucous membranes, and FSA is the fractional surface area during the hand-to-mouth contact. The surface transmission efficiency for Coliphage MS2 was adopted in this model because it is often used to represent respiratory virus in survival experiments and is recommended when similar data on SARS-CoV-2 is missing (3).

Finally, adding the estimated exposure dose (d_{air} or d_{surf}) into the dose-response model (Equation [4]), we get the probability of infection:

$$P_{inf} = 1 - e^{(-\frac{d}{k})} \qquad [4]$$

where P_{inf} is the probability of infection after a single exposure at dose d, d is the number of organisms inhaled (d_{air}) or contacted (d_{surf}) from equation [2] or [3], and k is the dose-response constant from Watanabe et al. (4).

Table S1. A summary of all air and surface samples collected from August 2020 to April 2021.

(a) Fall 2020

Sample Locations	Air Sample Time (minutes)	No. of Samples Positive/Total (%)		Surface Touch Points
		Aerosol	Surface	
Gym weight rooms	264±18	2/23 (8.7)	3/76 (3.9)	Drinking fountain*, pull down bars, weight pins, weight bars, dumbbells, barbells, equipment handles, floor*.
Office				
Hallway	323±61	0/13	0/12	Door handles
Mailroom	313±60	0/13	0/0	NA
Laboratory	NA	0/0	1/12 (8.3)	Door handle*, keyboard*, analyzer*, sink top*, light switch*.
Lunchroom	NA	0/0	0/10	Light switch, microwave, fridge handles, dining tables.
Restrooms	NA	0/0	0/12	Door handles, faucets, vanity tops, soap dispenser button.
Dining hall				
Food Line	345±78	0/11	0/0	NA
Entrance	359±82	0/12	0/31	Front desk, POS machines, west entrance handle, card swipe, railings at the waiting line and at the stairs
Musical complex				
Rehearsal rooms	259±45	0/11	0/16	Door handle, light switch, music stands, chairs, piano.
Performance halls	207±97	0/22	0/45	Light switch, door handles, music stands, piano, railings, seats.
Teaching complex				
Lobby	300±39	0/12	0/12	Door handles, floor.
Restrooms	273±60	0/21	0/33	Sink top, towel knob, soap dispenser, stall handles, floor.
Classrooms	277±60	0/14	1/32 (3.1)	Mouse, keyboard, desks*, door handles, railings.
Mechanical room[a]	256±77	0/9	0/0	NA
Student activity building				
Building entrance	240±8	0/7	0/9	Door handles, stair railings, sanitizer pump, floor
Common area	513±165	0/5	0/4	Random table and chair, exit sanitizer pump, end table, piano keys.
School buses	70±22	1/12 (8.3)	1/20 (5.0)	Handles*, rails*, stop strings*, seat backs*, hold straps*, sanitizer, driver's wheel, doorknob, task screen.
Total		3/185 (1.6)	6/328 (1.8)	

* indicating positive surface touchpoint

[a] Air samples were taken from the HVAC system tunnels.

(b) Winter 2021

Sample Locations	Air Sample Time (minutes)	No. of Samples Positive/Total (%)		Surface Touch Points
		Aerosol	Surface	
Gym weight rooms	307±71	1/48 (2.1)	1/143 (0.7)	Drinking fountain*, pull down bars, weight pins, weight bars, dumbbells, barbells, equipment handles.
Office				
Hallway	333±57	0/6	0/6	Door handles
Mailroom	328±61	0/6	0/0	NA
Laboratory	NA	0/0	0/6	Door handle, keyboard, analyzer, sink top, light switch.
Lunchroom	NA	0/0	1/6 (17.6)	Microwave*, fridge handles*, dining tables*.
Restrooms	NA	0/0	0/6	Door handles, faucets, vanity tops, soap dispenser button.
School buses	53±10	0/11	0/22	Handles, rails, stop strings, seat backs, hold straps, sanitizer.
Total		1/71 (1.4)	2/189 (1.1)	

* indicating positive surface touchpoint

Table S2. Mitigation strategies corresponding to each sampling location during study periods.

Sampled Area	Administrative Control			PPE		Other Location-specific Strategies
	Screening and Testing	Limited Occupancy	Physical Distancing	Cleaning and Disinfection	Require Masks	
Office						
Hallway	Y	I	Y	Y	Y	N
Mailroom	Y	Y	Y	Y	Y	N
Laboratory	Y	Y	Y	Y	Y	N
Lunchroom	Y	Y	Y	Y	Y	N
Restrooms	Y	Y	Y	Y	Y	N
Teaching complex						
Lobby	Y	I	Y	Y	Y	N
Restrooms	Y	Y	Y	Y	Y	N
Classrooms	Y	Y	Y	Y	Y	N
Mechanical room[a]	Y	I	N	N	Y	N
Student activity building						
Building entrances	Y	I	Y	Y	Y	N
Open area	Y	I	Y	Y	Y	N
Dining hall						
Hot food stand	Y	I	Y	Y	Y	Single direction traffic flows.
Entrance	Y	I	Y	Y	Y	Single direction traffic flows.
Musical complex						
Rehearsal studios	Y	Y	Y	Y	N	15-30min break between practices.
Performance halls	Y	Y	Y	Y	N	Designated spot spacing > 6ft.
Gym						
Weight rooms	Y	Y	Y	Y	Y	No partner workouts, extra fans for ventilation, only 1hr-appointment per person per day.
School bus						
Passengers' seats	N	Y	Y	Y	Y	Open windows, routes were < 15min.

Note: N: not required; Y: yes; I: indirect control by limiting card access to buildings, requiring 6ft spacing between individuals and/or furnishes.

Table S3. Primer-probe Sets Information

Assay	Target Gene	Primer/Probe	Concentration (nM)	Oligonucleotide Sequencea
N1	Nucleocapsid (N)	2019-nCoV_N1-F	200	5'-GACCCCAAAATCAGCGAAAT-3'
N1	Nucleocapsid (N)	2019-nCoV_N1-R	200	5'-TCTGGTTACTGCCAGTTGAATCTG-3'
N1	Nucleocapsid (N)	2019-nCoV_N1-P	200	5'-FAM-ACCCCGCATTACGTTTGGTGGACC-ZEN/Iowa Black-3'

Reference: 2019-Novel coronavirus (2019-nCoV) real-time quantitative rRT-PCR panel primers and probes. US Centers for Disease Control and Prevention [accessed 24 March 2020]

Table S4. Parameters used in the QMRA models

Parameter	Meaning	Value	Units	Sources and Notes
$gc:inf$	Gene copies to infectivity ratio	80	gc/PFU	SARS-CoV-2 isolate (hCoV-19/Netherlands/Zuid-Holland_10003/2020) was used to characterize the infection fraction (1).
eff_{air}	SASS 2300 recovery efficiency	0.48±0.10	unitless	Recovery rate of SASS 2300 was determined using bacteriophage MS2 in aerosols (5).
eff_{surf}	Swab recovery efficiency	0.51±0.13	unitless	Recovery rate of 3M™ Quick Swab 6432 was tested with human coronavirus OC43 in this study.
TE_{sh}	Transfer efficiency from surface to fingers	Metal: 0.37±0.16 Plastic: 0.80±0.21 Mixed: 0.59±0.28	unitless	Transfer of coliphage MS2 from surface to fingers at a relative humidity of 40% - 65% (6), the mixed surface value distribution was reconstructed with Monte-Carlo simulation[b].
TE_{hm}	Transfer efficiency from hand to mouth	0.20±0.06	unitless	Transfer of bacteriophage MS2 from finger to saliva (7).
FSA	Fractional surface area	4.5	cm^2	Assuming single partial finger immersion, we use the left-and-right-hands averaged median fraction (8) divided by 5, then times gender averaged adults hand surface area[c].
IR	Inhalation rate	Passenger:12.7±2.5 Workout: 91.8±15	L/min	Used minute ventilation for bus passengers (9). Workout data represent moderate- to high- intensive interval training workout among people in their 20s (10,11).
t	Stay duration	Passenger: 7.5±2 Workout: 40±10	min	Observed in this study.
k	Dose-response parameter	410	PFU	Model data were from infection of transgenic mice susceptible to SARS-CoV-1 and of mice with murine hepatitis virus strain 1 (MHV1) (4).

[b] We used Monte-Carlo simulation to randomly draw 10,000 observations from the plastic TE_{sh} and the metal TE_{sh} distributions, respectively, then combine the two 10,000 observations as the mixed surface TE_{sh} distribution. All distributions are considered normal distribution.

[c] U.S. EPA. Exposure Factors Handbook 2011 Edition (Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011.

Figure S1. The standard curve of quantitative rRT-PCR on SARS-CoV-2 virus synthetic fragment by primer set 1. X-axis is the log 10 concentration of template RNA of SARS-CoV-2 virus. Y-axis is the amplification cycles. c1, c2 and c3 represent three replicates.

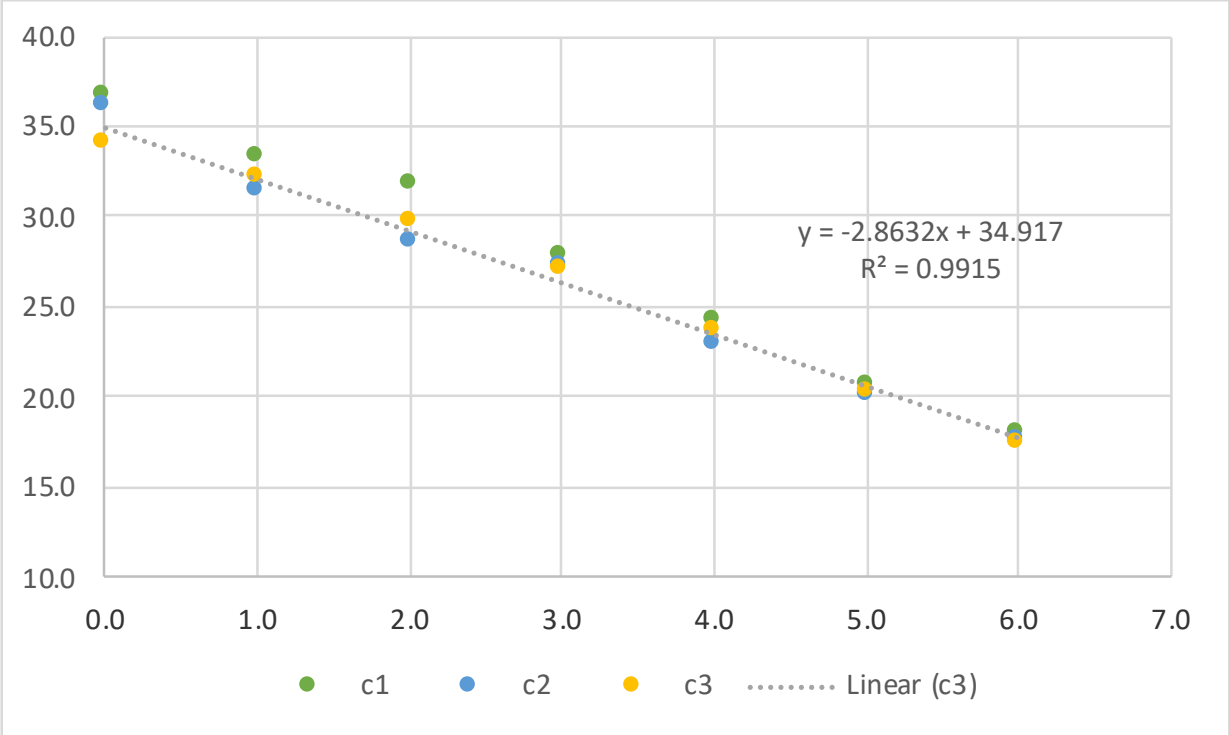
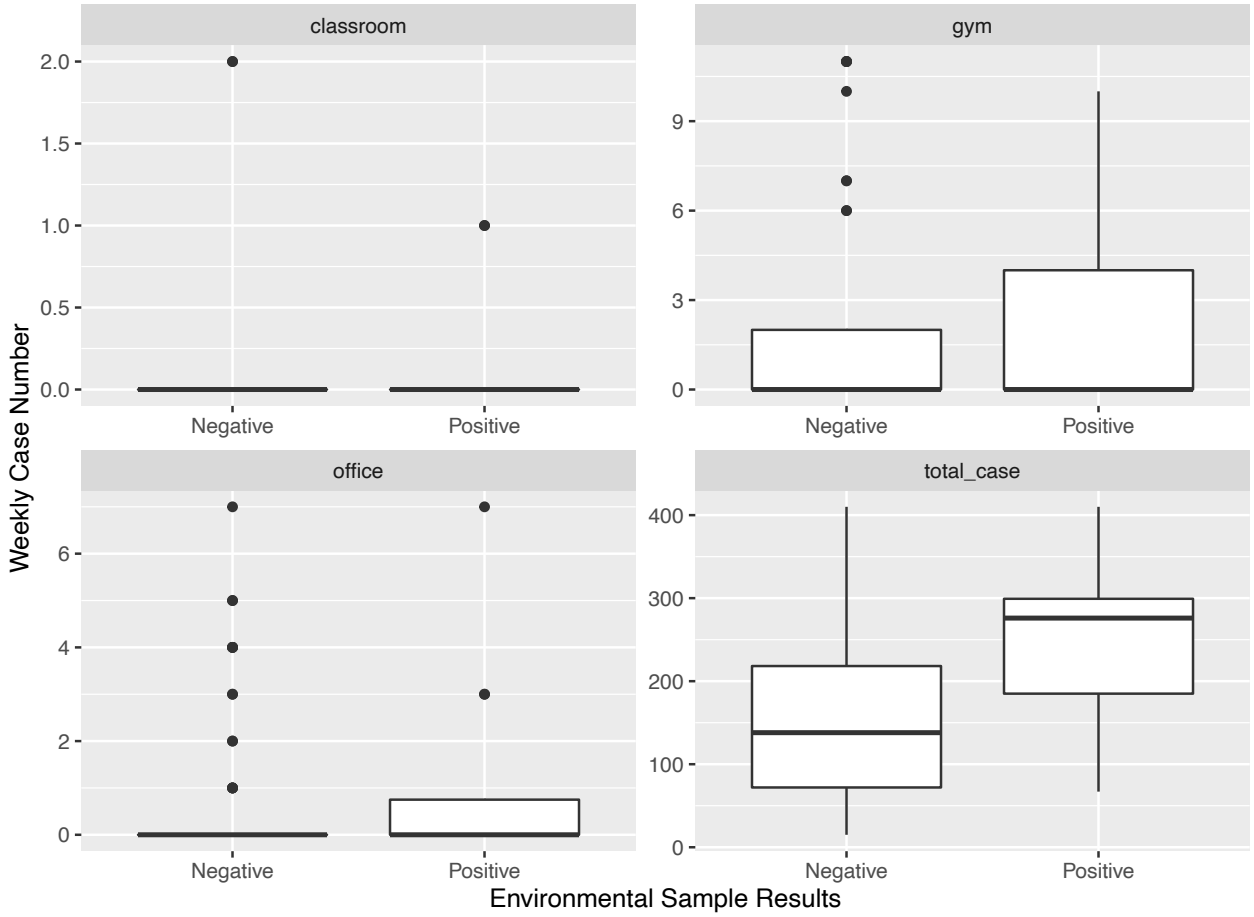
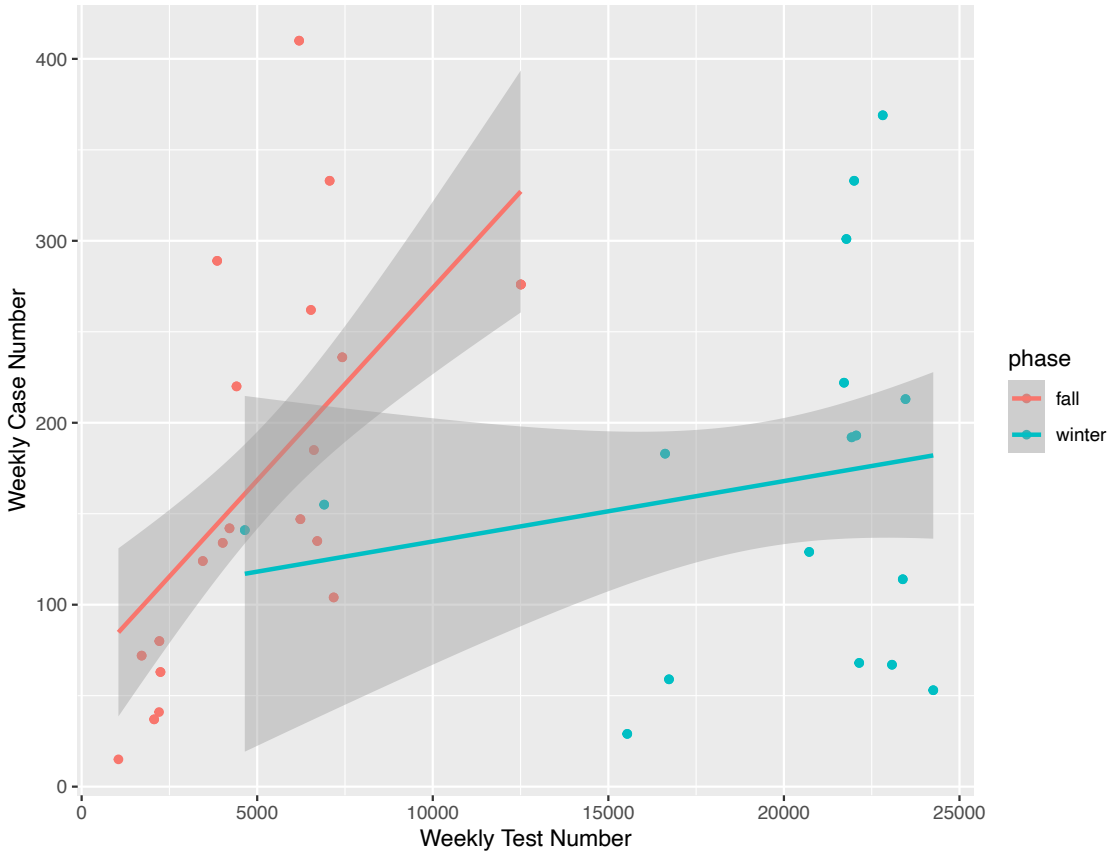


Figure S2. Distribution of case numbers between two sample groups or two study phases. (a) The lower and upper hinges of the box plots correspond to the 25th and 75th percentiles, the middle line equals to mean value, points are outliners. (b) The grey zone is the 95% confidence level interval for predictions from the linear models.

(a) Sample Results in Relationship with Weekly Case Numbers in Various Locations. Note: middle line is the mean value.



(b) The Relationship of Weekly Testing Capacity with Campus Case Number



References

1. Schijven J, Vermeulen LC, Swart A, Meijer A, Duizer E, de Roda Husman AM. Quantitative microbial risk assessment for airborne transmission of sars-cov-2 via breathing, speaking, singing, coughing, and sneezing. Environmental Health Perspectives. 2021;129(4):47002.
2. Gholipour S, Mohammadi F, Nikaeen M, Shamsizadeh Z, Khazeni A, Sahbaei Z, et al. COVID-19 infection risk from exposure to aerosols of wastewater treatment plants. Chemosphere [Internet]. 2021/02/01. 2021;273:129701. Available from: https://www.ncbi.nlm.nih.gov/pubmed/33517118
3. Harvey AP, Fuhrmeister ER, Cantrell ME, Pitol AK, Swarthout JM, Powers JE, et al. Longitudinal Monitoring of SARS-CoV-2 RNA on High-Touch Surfaces in a Community Setting. Environmental Science and Technology Letters. 2021;8(2):168–75.
4. Watanabe T, Bartrand TA, Weir MH, Omura T, Haas CN. Development of a dose-response model for SARS coronavirus. Risk Analysis [Internet]. 2010;30(7):1129–38. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7169223/pdf/RISA-30-1129.pdf
5. Dybwad M, Skogan G, Blatny JM. Comparative testing and evaluation of nine different air samplers: end-to-end sampling efficiencies as specific performance measurements for bioaerosol applications. Aerosol Science and Technology. 2014;48(3):282–95.
6. Lopez GU, Gerba CP, Tamimi AH, Kitajima M, Maxwell SL, Rose JB. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. Applied and Environmental Microbiology

[Internet]. 2013;79(18):5728–34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3754157/pdf/zam5728.pdf

7. Pitol AK, Bischel HN, Kohn T, Julian TR. Virus Transfer at the Skin-Liquid Interface. Environmental Science and Technology. 2017;51(24):14417–25.

8. AuYeung W, Canales RA, Leckie JO. The fraction of total hand surface area involved in young children's outdoor hand-to-object contacts. Environmental Research. 2008;108(3):294–9.

9. Zuurbier M, Hoek G, Hazel P Van Den, Brunekreef B. Minute ventilation of cyclists, car and bus passengers: An experimental study. Environmental Health: A Global Access Science Source. 2009;8(1):1–10.

10. Cruz R, Alves DL, Rumenig E, Gonçalves R, Degaki E, Pasqua L, et al. Estimation of minute ventilation by heart rate for field exercise studies. Scientific Reports. 2020;10(1):1–7.

11. Marmett B, Pires Dorneles G, Böek Carvalho R, Peres A, Roosevelt Torres Romão P, Barcos Nunes R, et al. Air pollution concentration and period of the day modulates inhalation of PM2.5 during moderate- and high-intensity interval exercise. Environmental Research. 2021;194:110528.