Title

Wastewater and surface monitoring to detect COVID-19 in elementary school settings: The Safer at School Early Alert project

Authors

Fielding-Miller, Rebecca
Karthikeyan, Smruthi
Gaines, Tommi
et al.

Publication Date

2023-01-25

DOI

10.1101/2021.10.19.21265226

TITLE:

Wastewater and surface monitoring to detect COVID-19 in elementary school settings: The Safer at School Early Alert project

AUTHORS:

Rebecca Fielding-Miller[1,3,*], Smruthi Karthikeyan[2], Tommi Gaines[3], Richard S. Garfein[1], Rodolfo Salido[4], Victor Cantu[4], Laura Kohn[5], Natasha K Martin[3], Carrissa Wijaya[1], Marlene Flores[1], Vinton Omaleki[1], Araz Majnoonian[1,6], Patricia Gonzalez-Zuniga[1], Megan Nguyen[1,7], Anh V Vo[1,7], Tina Le[1], Dawn Duong[1], Ashkan Hassani[1], Austin Dahl[8], Samantha Tweeten[9], Kristen Jepsen[10], Benjamin Henson[10], Abbas Hakim[11], Amanda Birmingham[12], Adam M. Mark[12], Chanond A Nasamran[12], Sara Brin Rosenthal[12], Niema Moshiri[13], Kathleen M. Fisch[11,12], Greg Humphrey[2], Sawyer Farmer[2], Helena M. Tubb[2], Tommy Valles[2], Justin Morris[13,15], Jaeyoung Kang[14], Behnam Khaleghi[13], Colin Young[13], Ameen D Akel[16], Sean Eilert[16], Justin Eno[16], Ken Curewitz[16], Louise C Laurent[6], Tajana Rosing[13], SEARCH, Rob Knight[2,4]

AFFILIATIONS:

[1]University of California San Diego, Herbert Wertheim School of Public Health and Human Longevity Science

[2]University of California San Diego, Department of Pediatrics

[3] University of California San Diego, School of Medicine, Division of Infectious Disease and Global Public Health

[4] University of California San Diego, Department of Bioengineering

[5]Kohn education consulting

[6]University of California San Diego and San Diego State University Joint Doctoral Program in Public Health

[7] Johns Hopkins University Bloomberg School of Public Health, International Health Social and Behavioral Interventions

[8]Independent Consultant

[9]County of San Diego, Health and Human Services Agency

[10]University of California San Diego, Institute for Genomic Medicine

[11]University of California San Diego, Department of Obstetrics Gynecology and Reproductive Sciences

[12]University of California San Diego, Center for Computational Biology & Bioinformatics

[13]University of California San Diego, Department of Computer Science & Engineering

[14]University of California San Diego, Department of Electrical and Computer Engineering

[15]San Diego State University, Department of Electrical and Computer Engineering

[16]Micron Technology, Inc.

*Corresponding author: rfieldingmiller@health.ucsd.edu

Abstract:

Schools are high-risk settings for SARS-CoV-2 transmission, but necessary for children's educational and social-emotional wellbeing. While wastewater monitoring has been implemented to mitigate outbreak risk in universities and residential settings, its effectiveness in community K-12 sites is unknown. We implemented a wastewater and surface monitoring system to detect SARS-CoV-2 in nine elementary schools in San Diego County. Ninety-three percent of identified cases were associated with either a positive wastewater or surface sample; 67% were associated with a positive wastewater sample, and 40% were associated with a positive surface sample. The techniques we utilized allowed for near-complete genomic sequencing of wastewater and surface samples. Passive environmental surveillance can complement approaches that require individual consent, particularly in communities with limited access and/or high rates of testing hesitancy.

One sentence summary: Passive wastewater and surface environmental surveillance can identify up to 93% of on-campus COVID-19 cases in public elementary schools; positive samples can be sequenced to monitor for variants of concerns with neighborhood level resolution.

Main text:

Safely operating schools during the COVID-19 pandemic is a public health challenge. In schools, unvaccinated individuals spend extended amounts of time in close proximity, typically indoors. They are therefore potentially high-risk spaces for respiratory virus transmission. However, in-person schooling is essential for children's social, physical, and emotional wellbeing(*1*) and provides childcare essential for parental workforce participation. School closures resulting from the pandemic have caused high rates of job loss for female caregivers and households headed by single mothers are at significantly increased risk of falling into poverty due to school closures(*2-4*).

Implementing multiple overlapping interventions, including masking, improved ventilation, and symptom screening, can reduce viral transmission(*5*). Timely detection of infections to enable appropriate isolation of cases, and quarantine of exposed contacts is crucial for preventing in-school transmission that could lead to larger community outbreaks.

Effective vaccines are widely available to adults in high-income countries. However, in historically marginalized communities, structural barriers that inhibit access to diagnostic testing (i.e., medical mistrust, lack of paid time off, poor geographic access) also present barriers to vaccine uptake(*6-8*). Strategies to rapidly identify COVID-19 cases in communities with low testing and vaccine uptake are necessary to achieve health equity, reduce morbidity and mortality, and avoid the emergence of new variants of concern (VoCs) with increased vaccine escape potential(*9*).

Wastewater surveillance has gained attention as a tool for passive surveillance of community- and building-level SARS-CoV-2 infections in municipalities and universities(*10*). In California, a large residential university with free, university-mandated testing found that large-scale wastewater monitoring allowed the university to identify cases in specific campus buildings and residential halls. Notifying building occupants following a positive wastewater sample was significantly associated with increased diagnostic testing uptake compared to testing uptake prior to the notification, and 85% of all diagnosed infections among on-campus residents were detected in wastewater(*11*).

The passive nature of wastewater sampling is promising for school COVID-19 surveillance in communities where students, parents, and staff are more likely to face structural barriers to vaccination and diagnostic testing uptake. However, there are currently no data on the effectiveness of this approach in non-residential settings. Two concerns about potential effectiveness of wastewater sampling in these settings are that 1) not all individuals have daily bowel movements on site; and 2) spatial resolution is limited to entire buildings or building clusters because of sewer access locations.

We developed an environmental monitoring system that utilizes wastewater and daily surface sample surveillance to detect COVID-19 cases in elementary schools and childcare settings. We named the project Safer at School Early Alert (SASEA). SASEA consists of four primary components: (1) Daily environmental sampling for SARS-CoV-2 using wastewater from the whole site and surface swabs (typically the center of a classroom floor) from individual classrooms; (2) Rapid results reporting to site administrators (approximately 30 hours after sample collection); (3) On-site diagnostic testing of students and staff when SARS-CoV-2 was detected in wastewater or surface samples; and (4) Risk mitigation via environmental modification (e.g., moving classes outdoors, increasing ventilation in classrooms with a potential

case) and health communication messaging (e.g., encouraging double masking, recommending wider testing among household members) (Fig. 1). Surface sampling was included because although SARS-CoV-2 transmission though fomites is considered uncommon(*12*), we were able to recover traces of viral RNA by surface sampling of rooms occupied by infected individuals in a hospital setting(*13*), suggesting that surface sampling can provide a complementary approach to wastewater viral monitoring.

Figure 1 about here

We piloted SASEA in nine public elementary schools in San Diego County during the 2020-2021 academic year. Pilot sites were selected from ZIP Codes with COVID-19 rates above the county median and with high levels of social vulnerability according to the California Healthy Places Index (HPI)(*14*). During a 12-week validation phase (November 16 - March 1, 2021), we conducted daily wastewater monitoring at each site and surface sampling in each classroom where children were present. We also provided weekly diagnostic testing for all consenting students and staff on campus to validate the environmental monitoring system. The County of San Diego, Health and Human Services (SDHHSA) agency shared de-identified data for all cases that had been present at any of our pilot sites. We worked with school principals and COVID-19 liaisons to match each case to a classroom. School administrators provided additional information about cases that were reported to them.

Wastewater autosamplers were deployed at each site to collect time-weighted composite samples and programmed to sample every 10-15 minutes over a 7h interval. Sites were sampled each day that children were present. Autosamplers were deployed above ground at sewer cleanouts and manholes. To support the high volume and rapid turnaround necessary for this project, a streamlined, high-throughput wastewater processing pipeline was implemented(*15*). Further details on wastewater sampling methodology are available elsewhere(*15*, *16*).

Surface samples were collected daily from all classrooms containing stable cohorts of children (i.e., classrooms used for brief one-on-one services for students were excluded). Classroom teachers and/or custodial staff swabbed a one-foot square area in the center of each classroom floor at the end of each day prior to classroom cleaning. Floors were chosen as the monitored surface because they were found to have the highest prevalence of SARS-CoV-2 detection among surfaces examined in COVID-19 patient hospital rooms(*17*). Additional details about surface sampling methodology and technical performance are available elsewhere(*18*). The principal and COVID-19 liaison were notified of wastewater or surface sample results by email (typical turnaround timebetween 26 and 36 hours). All sites were given template language to notify staff and parents of positive results, although sites chose to implement these notifications in a variety of ways. Sites were also given educational materials on contact tracing, diagnostic testing access, and supportive services available for individuals who tested positive.

Onsite diagnostic testing required informed consent, which was obtained continuously throughout the study. Study consent, and hence the number of individuals participating in weekly testing, rose steadily throughout the 12-week study period. By the end of the study period, 1,294 (75.4%) of the 1,717 individuals consistently present at the sites had consented to onsite diagnostic testing, of which 1,275 (98.5%) were tested at least once.

There were 447 data collection days across the nine sites (i.e., approximately 50 school days per site over the 12-week study period). In this period, SARS-CoV-2 was detected in 374 surface samples and 133 wastewater samples. Eighty-nine individuals tested positive; 42 via onsite

testing and 47 through outside testing. We do not have data on the number of outside tests among students or staff that received negative results. We observed only two instances of likely in-class transmission in which 3 or more individuals in the same classroom tested positive within a 14-day period. Both instances were later confirmed as outbreaks by the County of San Diego, Health and Human Services Agency.

Of the 89 identified on-campus cases, 83 (93%) were associated with a positive wastewater or same-room surface sample in the 7-day window preceding the individual's last day on campus (95% CI: 88% - 98%). The majority of these, 68 (76%), were associated with a positive wastewater sample (95% CI: 68% - 75%). Of the 72 identified cases among individuals associated with a single classroom, 29 (40%) corresponded with a positive surface sample in the associated room in the 7-day window preceding the individual's last day on campus (95% CI: 29% - 52%).

Positive surface or wastewater signals occurred on 240 (60%) of study days, during which 76 (28%) days had a positive wastewater and surface signal on the same day. Just under half (47%, n=127) of days with positive signals were associated with a diagnosed case in the 7-day window following the signal (95% CI: 41% - 52%). Seventy (53%) of positive wastewater signals were followed by an identified case within 7 days (95% CI: 44% - 61%), while 40 (11%) of positive surface samples were followed by a detected case within 7 days (95% CI: 8% - 14%).

Consent to onsite testing rose steadily through the validation phase. By week 9, we had obtained consent for 70% of eligible students and staff across all sites. In weeks 9-12, there were 157 positive surface samples, 67 positive wastewater samples, and 19 identified cases, 15 of which were associated with a classroom (the remaining 4 cases were among non-classroom staff members). Nine of the cases (60%) linked to a classroom were associated with a positive surface sample (95% CI: 36% - 85%) and 18 identified cases (95%) were associated with a positive wastewater sample (95% CI: 85% - 100%). In the same time-period, positive wastewater or surface samples occurred on 130 days and 44 (34%) of these were associated with an identified case these (95% CI: 26% - 42%). All 19 cases (100%) identified in this time period were associated with either a surface sample, wastewater sample, or both (figure 2).

Figure 2 about here

Testing uptake within SASEA partner schools was higher than in nearby districts. In February of 2021, approximately 13% of onsite students and staff in a large local district accessed onsite COVID-19 tests(*19*), compared to 78% of students and staff across all SASEA partner sites in the same time period. Five schools achieved consent rates over 90%, and at 3 sites 100% of all on-campus staff and students had consented to weekly testing by the end of the validation period (figure 3). While these findings are consistent with other settings where environmental monitoring notifications have been shown to increase diagnostic testing uptake(*11*), more research is needed to understand how other aspects of our study design, including the requirement to consent to testing as part of an ongoing research process, recruitment strategies and school administrator involvement, influenced school-wide testing rates.

Figure 3 about here

Our findings suggest that environmental surveillance via wastewater and surface sampling can be an effective passive screening tool to complement and potentially enhance individual testing approaches. We found high concordance between diagnosed cases and positive environmental

samples, with lower concordance between positive environmental samples and diagnosed cases. Diagnostic testing consent is crucial for system effectiveness; however, it is important to acknowledge that parents and staff may not consent to onsite diagnostic testing for a wide array of reasons. Positive environmental signals should prompt the increased use of risk mitigation measures (i.e., masking/double-masking, social distancing, ventilation) while waiting for responsive testing implementation and results, and/or in the absence of identifying a case.

While wastewater detection of SARS-CoV-2 viral RNA via PCR-based approaches is valuable in tracking the viral prevalence in a given community, viral genome sequencing of positive wastewater samples can help elucidate strain geospatial distributions, thereby aiding in identification of outbreak clusters and tracking of prevailing/newly emerging variants. The methods used in this study to detect SARS-CoV-2 viral RNA allowed us to sequence viral genomes directly from the wastewater to characterize the circulating viral variants/lineages. This technique may have applications in areas where sequencing capacity and/or individual diagnostic testing uptake is limited. Sequencing of the environmental samples enabled recovery of near complete SARS-CoV-2 genomes (>99% genome coverage) from wastewater samples with cycle threshold values as high as 37.6 using a miniaturized tiled amplicon sequencing approach(*16*). In this study, 64 of 133 positive wastewater samples yielded near-complete SARS-CoV-2 viral genomes (average genome coverage of 93.2%). All positive surface samples submitted for tiled amplicon sequencing (n=10) generated near-complete viral genomes (genome coverage > 98%). One SARS-CoV-2 genome sequenced from a carpeted floor surface was associated with a genome from a SASEA clinical testing sample via clustering in a phylogenetic tree (figure 4). The individual whose diagnostic sample was sequenced was confirmed to have been present in the classroom with a positive surface sample, demonstrating the utility of surface sampling to identify potential COVID-19 cases with higher spatial resolution than wastewater monitoring alone. Similarly, SARS-CoV-2 genomes sequenced from wastewater can be associated to nasal samples via clustering, while consecutive observations of specific genomes in wastewater suggest persistent viral shedding.

Among the sequenced samples, we identified the Alpha variant (B.1.1.7) in 14.2% of the wastewater samples and 8.6% of SASEA diagnostic tests (nasal swabs,; and the Epsilon variant (B.1.427/B.1.429) in 22.2% of wastewater samples, 25.0% of SASEA clinical tests, and 10.0% of surface samples (figure 4). When sequencing data was available for both environmental monitoring modalities with matched spatial-temporal characteristics (same school, 5-day time window), we were able to match strain identifications between surface and wastewater positive samples. Notably, the Alpha variant was detected in a January 11th wastewater sample, 25 days after it was first identified in the region(*20*). Following the pilot phase, the Delta variant (B.1.617.2) was identified at a school site on April 26, 15 days after it was first identified in the county via diagnostic testing.

Figure 4 about here

While the SDHHSA provided data on cases affiliated with SASEA partner sites, our denominator only includes known campus-associated cases. We lack data on cases that may have contributed to surface or wastewater samples during our 12-week validation phase but did not test through SASEA or an agency that reports to SDHHSA. San Diego has close social and economic links with Tijuana, Mexico and four of our partner schools were within 10 miles of the San Ysidro Port of Entry. SDHHSA authorities are notified of all cases in other counties or countries (i.e., Mexico) that provide a residential address in San Diego County. However, it is

possible that some individuals may have tested elsewhere without providing a residential address in the county. It is also possible that individuals may have tested positive using an at-home test kit and declined to notify SDHHSA of positive results. We also do not have data on individuals who tested and received negative results through outside providers or at home test kits during our 12-week pilot phase. For these reasons we are unable to calculate the true sensitivity and specificity of the environmental monitoring system. However, to our knowledge, ours is the only systematic investigation of the accuracy of environmental monitoring to detect and rapidly respond to cases in school sites.

The SASEA system was designed for communities that face social and structural barriers to diagnostic testing and vaccine access. Wastewater and surface sample monitoring are anonymous, aggregate, and can provide sentinel surveillance for early detection of outbreaks and VoCs. Even in the absence of a diagnosed case, positive environmental samples serve as a behavioral cue to increase or re-implement risk mitigation measures in a classroom or entire school. Since neighborhood public schools serve a specific geographic region, environmental monitoring has the additional benefit of acting as an early warning system for the local community from which students are drawn. Utilizing public schools as community sentinel surveillance sites for SARS-CoV-2 can support community-tailored interventions such as ensuring materials are translated into languages spoken in the community, working with field staff to ensure culturally competent outreach, and providing community-specific testing and vaccine clinics.

REFERENCES

1. N. Kalluri, C. Kelly, A. Garg, Child care during the COVID-19 pandemic: A bad situation made worse. *Pediatrics* **147**, (2021).
2. R. J. Petts, D. L. Carlson, J. R. Pepin, A gendered pandemic: Childcare, homeschooling, and parents' employment during COVID□19. *Gender, Work & Organization*, (2020).
3. M. I. Cardel, N. Dean, D. Montoya-Williams, Preventing a secondary epidemic of lost early career scientists. Effects of COVID-19 pandemic on women with children. *Annals of the American Thoracic Society* **17**, 1366-1370 (2020).
4. T. Alon, M. Doepke, J. Olmstead-Rumsey, M. Tertilt, "The impact of COVID-19 on gender equality," (National Bureau of economic research, 2020).
5. J. Lessler *et al.*, Household COVID-19 risk and in-person schooling. *Science* **372**, 1092-1097 (2021).
6. L. Doornekamp, L. van Leeuwen, E. van Gorp, H. Voeten, M. Goeijenbier, Determinants of Vaccination Uptake in Risk Populations: A Comprehensive Literature Review. *Vaccines* **8**, (2020).
7. C. Dodds, I. Fakoya, Covid-19: ensuring equality of access to testing for ethnic minorities. *BMJ* **369**, m2122 (2020).
8. N. E. MacDonald, R. Butler, E. Dube, Addressing barriers to vaccine acceptance: an overview. *Human Vaccines & Immunotherapeutics* **14**, 218-224 (2018).
9. A. Fontanet *et al.*, SARS-CoV-2 variants and ending the COVID-19 pandemic. *The Lancet* **397**, 952-954 (2021).
10. F. Hassard, L. Lundy, A. C. Singer, J. Grimsley, M. Di Cesare, Innovation in wastewater near-source tracking for rapid identification of COVID-19 in schools. *The Lancet Microbe* **2**, e4-e5 (2021).
11. S. Karthikeyan *et al.*, Rapid, large-scale wastewater surveillance and automated reporting system enabled early detection of nearly 85% of COVID-19 cases on a University campus. *medRxiv*, (2021).
12. E. A. Meyerowitz, A. Richterman, R. T. Gandhi, P. E. Sax, Transmission of SARS-CoV-2: A Review of Viral, Host, and Environmental Factors. *Ann Intern Med* **174**, 69-79 (2021).
13. C. Marotz *et al.*, SARS-CoV-2 detection status associates with bacterial community composition in patients and the hospital environment. *Microbiome* **9**, 132 (2021).
14. D. W. Delaney T., Dowling H., Naizlish N., Chapman D., Hill L, Orndahl C., Sabo R., Snellings L., Blackburn SS., Woolf S. , "Healthy Places Index (HPI 2.0)," (Public Health Alliance of Southern California, 2018).
15. S. Karthikeyan *et al.*, High-Throughput Wastewater SARS-CoV-2 Detection Enables Forecasting of Community Infection Dynamics in San Diego County. *mSystems* **6**, e00045-00021 (2021).

16. G.-B. L. Sheikhzadeh CH., Adams AM., Ott O., Karthikeyan S., Marotz L., Humphrey G. (Knight Lab WBE, 2021).

17. C. Marotz *et al.*, SARS-CoV-2 detection status associates with bacterial community composition in patients and the hospital environment. *Microbiome* **9**, (2021).

18. R. A. Salido *et al.*, Comparison of heat-inactivated and infectious SARS-CoV-2 across indoor surface materials shows comparable RT-qPCR viral signal intensity and persistence. *bioRxiv*, 2021.2007.2016.452756 (2021).

19. "San Diego Unified School District COVID-19 School Testing Results." *San Diego Unified School District*, San Diego, CA, 25 August 2021, https://web.archive.org/web/20210812134829/https://sandiegounified.org/covid-19_status/covidtesting

20. J. Mullen, Tsueng G., Latif AA., Alkuzweny M., Cano M., Haag E., Zhou J., Hufbauer E., Matteson N., Andersen K., Wu C., Su A., Gangavarpu K., Hughes L., Center for Viral Systems Biology. (2020).

21. Grubaugh, N. D. *et al.* An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol.* **20**, 8 (2019).

22. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).

23. Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* **5**, 1403–1407 (2020).

24. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018).

25. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).

26. Cantrell, K. *et al.* EMPress Enables Tree-Guided, Interactive, and Exploratory Analyses of Multi-omic Data Sets. *mSystems* **6**, (2021).

Acknowledgments:

We are deeply grateful to the leadership, staff, students, and families at our partner school districts and school sites across San Diego County. This project would not have been possible without their enthusiasm and generosity. We thank our colleagues at the County of San Diego, Health and Human Services Agency for their vision and support, particularly John Malone, Sarah Stous, and Rorick Luepton. At UCSD, Isabella Cuturrofo, Jeanessa Mendoza, Kristine Ngo, Jessica Ni, and Elizabeth Frost all provided vital support with project implementation. RFM thanks Ms. Esther Krohne for inspiring the project.

The opinions and assertions expressed herein are those of the authors and do not necessarily reflect the official policy or position of the County of San Diego Health and Human Services Agency

Funding:

County of San Diego, Health and Human Services Agency (RFM)

National Institutes of Health Grant K01MH112436 (RFM)

National Institute of Health Grant UL1TR001442

National Institute of Health Grant S10OD026929 (Jepsen)

NSF Grants Numbers 1730518, 1826967, 1659104, 2100237, 2112167, 2052809, 2028040 (Rosing)

Centers for Disease Control BAA Contract 75D30120C09795 (Andersen)

This work was supported in part by CRISP, one of six centers in JUMP, an SRC program sponsored by DARPA (Rosing)

Author Contributions:

RFM: Conceptualization; Methodology; Formal Analysis; Investigation; Writing - original draft; Visualization; Supervision; Funding Acquisition
SK: Methodology; Formal Analysis; Investigation; Data curation; Writing - original draft; Visualization
TG: Methodology; Formal Analysis; Writing - original draft.
RSG: Methodology; Data curation; Writing - original draft; Visualization; Supervision.
RS: Methodology; Formal Analysis; Investigation; Writing - original draft.
VC: Methodology; Formal Analysis; Investigation; Data curation; Writing - original draft.
LK: Conceptualization; Methodology; Investigation; Data curation; Writing - original draft; Supervision; Project administration; Funding Acquisition
NKM: Methodology; Writing - original draft.
CW: Supervision; Project administration.
MF: Investigation; Data curation.
VO: Investigation; Data curation.
AM: Investigation; Data curation.
PGZ: Investigation; Supervision.
MN: Investigation; Data curation.
AVV: Investigation; Data curation; Visualization

TL: Investigation.
DD: Investigation.
AH: Investigation.
ST: Resources; Supervision; Project administration.
KJ: Methodology; Investigation; Resources.
BH: Methodology; Investigation.
AH: Methodology; Investigation.
AB: Methodology; Investigation.
AMM: Methodology; Investigation.
CAN: Methodology; Investigation.
SBR: Methodology; Investigation.
NM: Methodology; Investigation.
KMF: Methodology; Investigation.
GH: Investigation; Data curation.
SF: Investigation; Data curation.
HMT: Investigation; Data curation.
TV: Investigation; Data curation.
JM: Investigation; Data curation.
JK: Investigation; Data curation.
BK: Investigation; Data curation.
CY: Investigation; Data curation.
ADA: Investigation; Data curation.
SE: Investigation; Data curation.
JE: Investigation; Data curation.
KC: Investigation; Data curation.
LCL: Resources
TR: Investigation; Data curation.
RK: Conceptualization; Methodology; Resources; Writing - original draft; Supervision.

Competing interests: Authors declare they have no competing interests.

Data and materials availability: School-level data are available from the first author on reasonable request. All wastewater and nasal-swab sequencing data have been deposited to GISAID and their accession details provided in Supplemental File Data S1

List of supplementary materials:

Methods
Data S1: Wastewater and nasal-swab sequencing data GISAID accession details

Fig 1: Safer at School Early Alert (SASEA) system

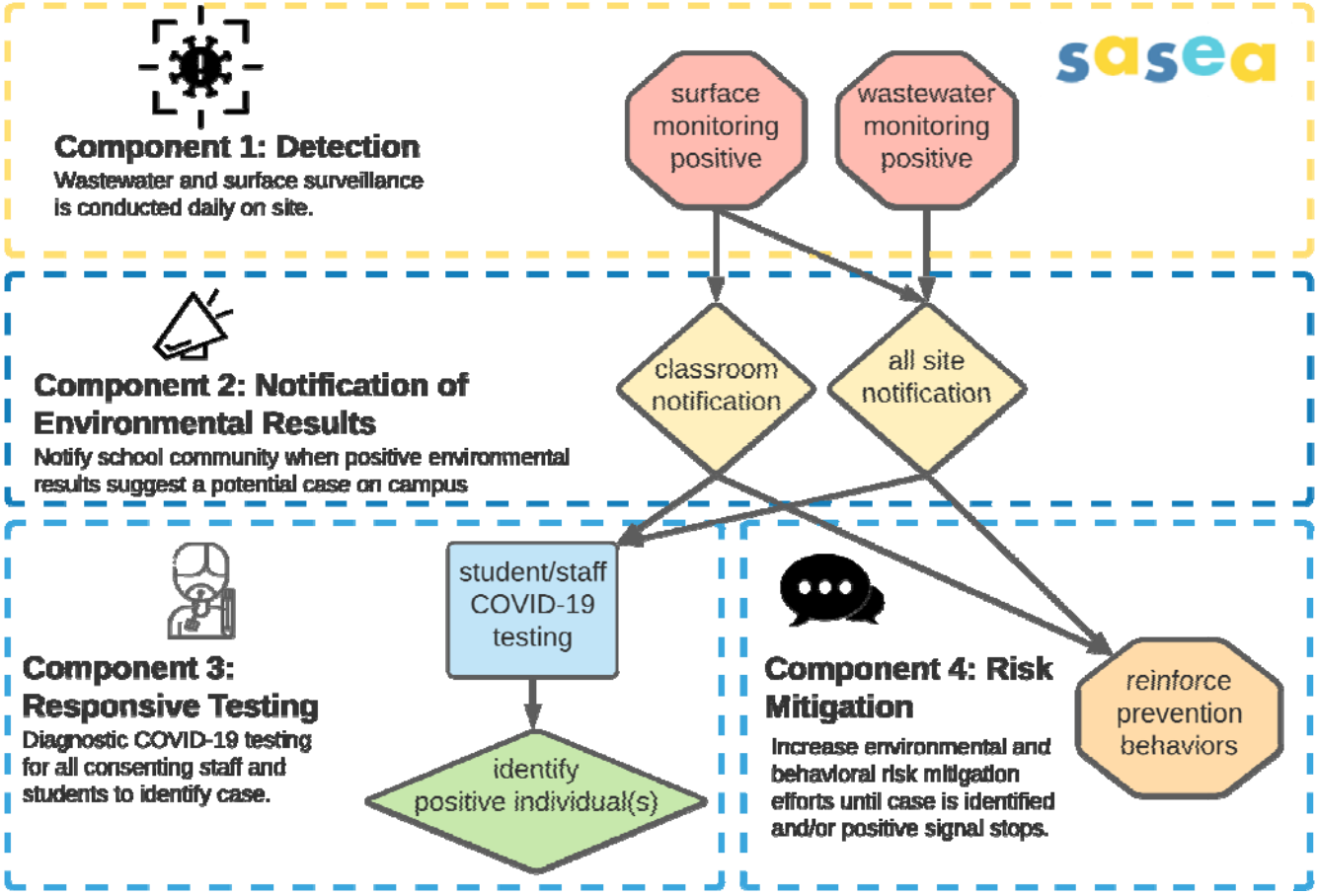


Figure 1: Safer at School Early Alert (SASEA) system

Fig 2: Wastewater and surface sampling and 95% confidence interval across full 12-week pilot period, and with consent at 70% or above (weeks 9-12)

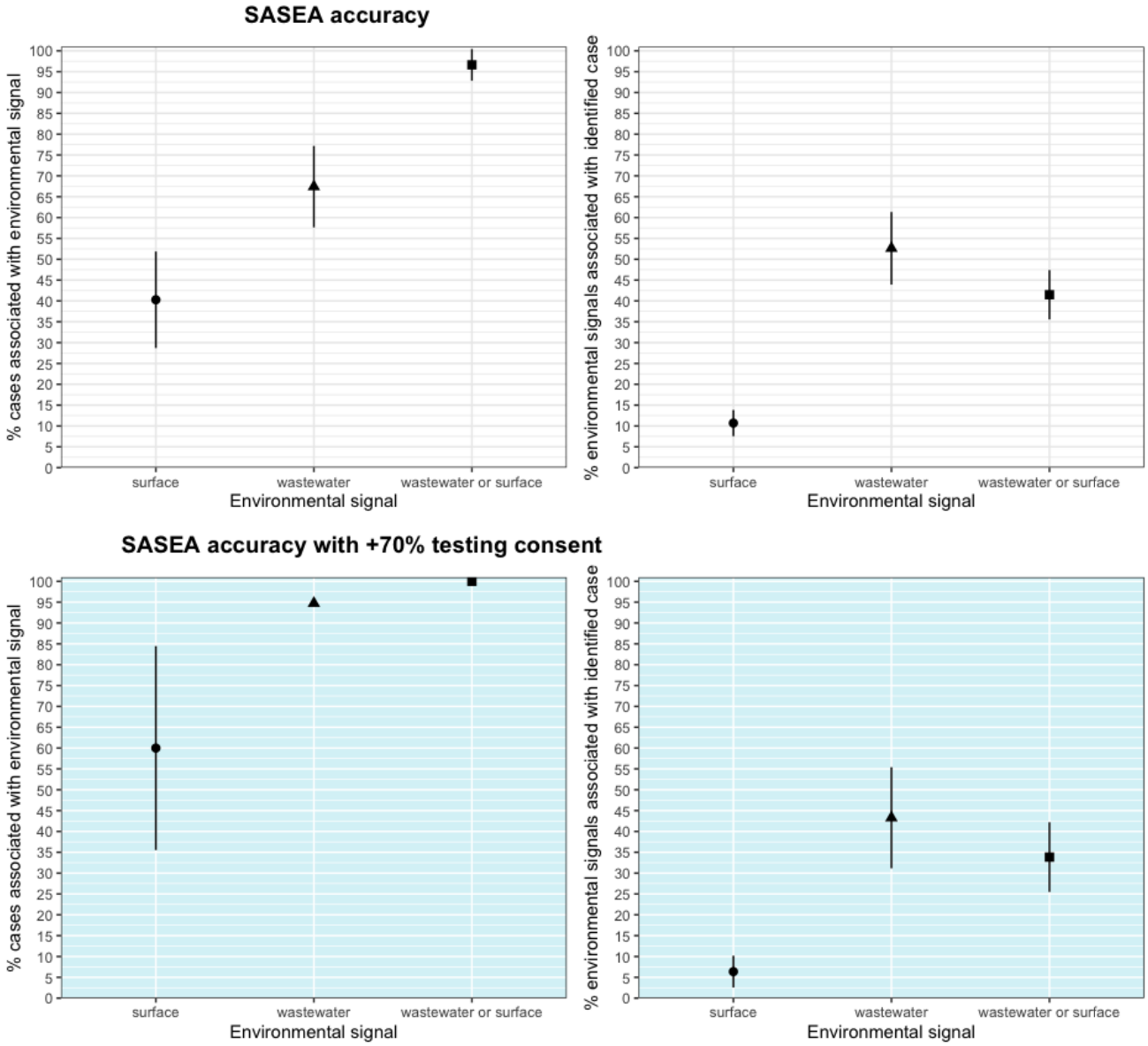


Fig 3: Individual diagnostic testing consent over time by site (gray) and total across all 9 sites (red) throughout 12-week pilot phase

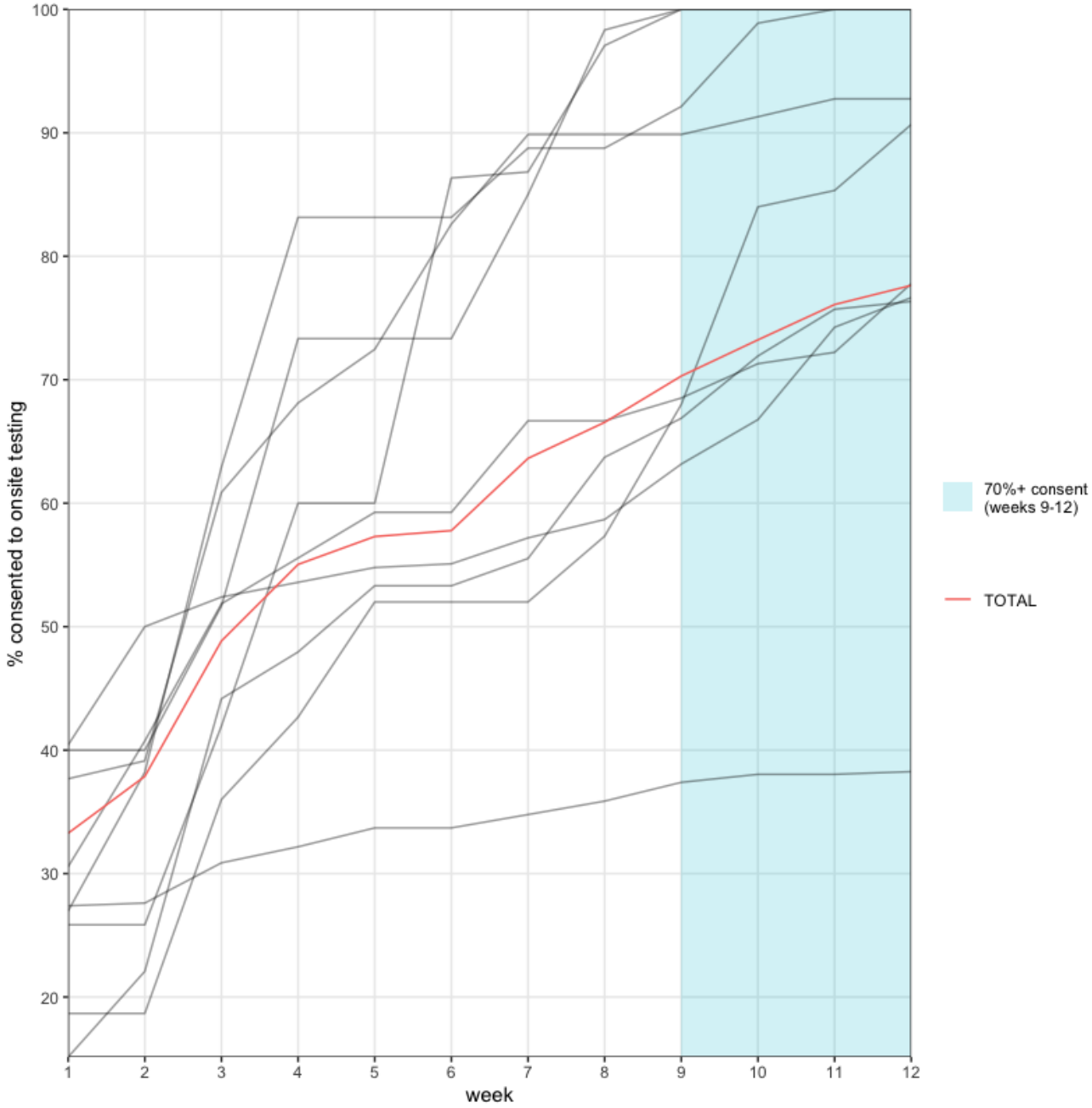
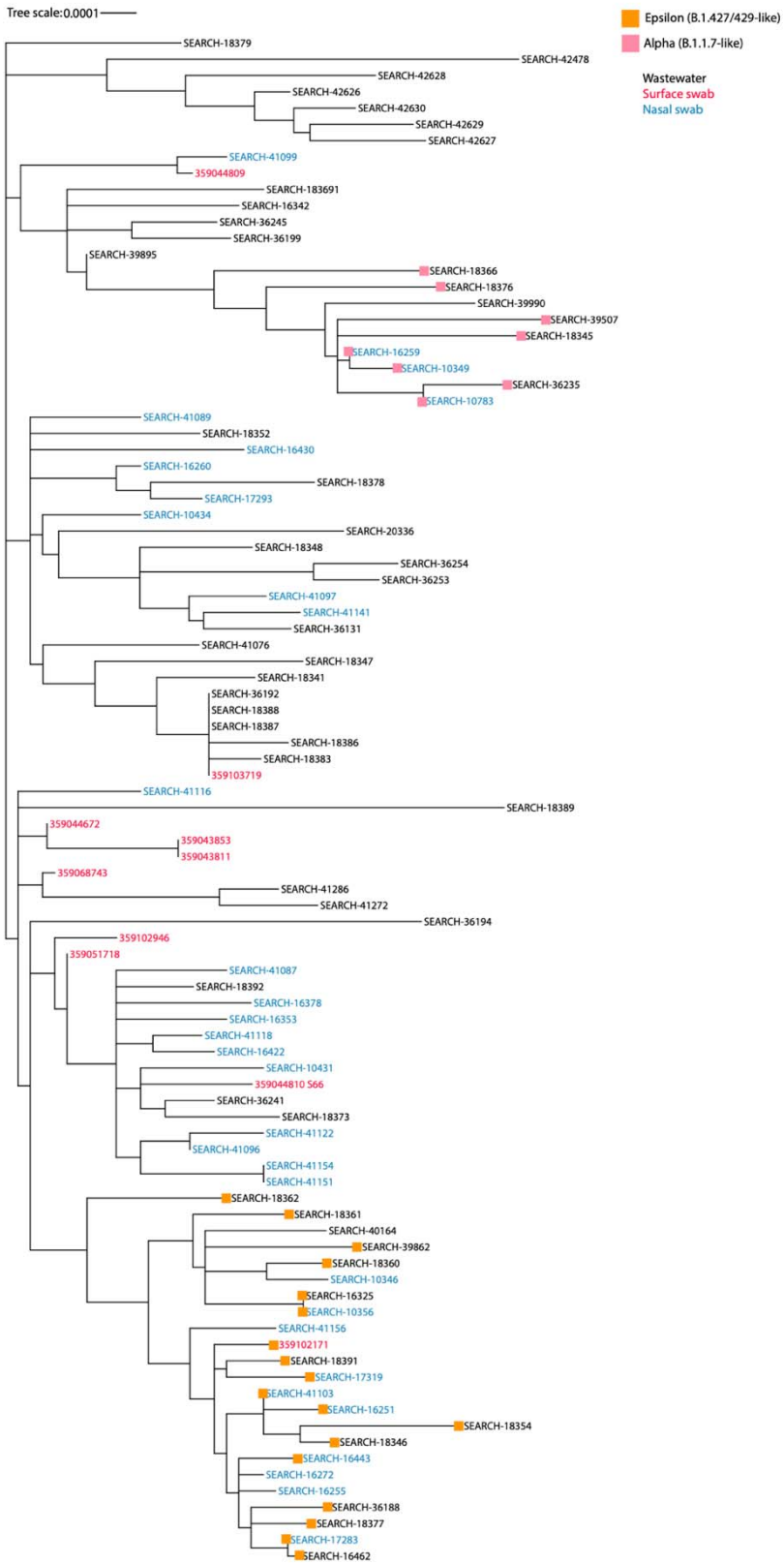


Figure 4: Genomic sequencing



Supplementary Materials for

Wastewater and surface monitoring to detect COVID-19 in elementary school settings: The Safer at School Early Alert project

AUTHORS:
Rebecca Fielding-Miller[1,3,*], Smruthi Karthikeyan[2], Tommi Gaines[3], Richard S. Garfein[1], Rodolfo Salido[4], Victor Cantu[4], Laura Kohn[5], Natasha K Martin[3], Carrissa Wijaya[1], Marlene Flores[1], Vinton Omaleki[1], Araz Majnoonian[1,6], Patricia Gonzalez-Zuniga[1], Megan Nguyen[1,7], Anh V Vo[1,7], Tina Le[1], Dawn Duong[1], Ashkan Hassani[1], Samantha Tweeten[8], Kristen Jepsen[9], Benjamin Henson[9], Abbas Hakim[10], Amanda Birmingham[11], Adam M. Mark[11], Chanond A Nasamran[11], Sara Brin Rosenthal[11], Niema Moshiri[12], Kathleen M. Fisch[10,11], Greg Humphrey[2], Sawyer Farmer[2], Helena M. Tubb[2], Tommy Valles[2], Justin Morris[12,14], Jaeyoung Kang[13], Behnam Khaleghi[12], Colin Young[12], Ameen D Akel[15], Sean Eilert[15], Justin Eno[15], Ken Curewitz[15], Louise C Laurent[6], Tajana Rosing[12], SEARCH, Rob Knight[2,4]

AFFILIATIONS:
[1]University of California San Diego, Herbert Wertheim School of Public Health and Human Longevity Science
[2]University of California San Diego, Department of Pediatrics
[3] University of California San Diego, School of Medicine, Division of Infectious Disease and Global Public Health
[4] University of California San Diego, Department of Bioengineering
[5]Kohn education consulting
[6]University of California San Diego and San Diego State University Joint Doctoral Program in Public Health
[7] Johns Hopkins University Bloomberg School of Public Health, International Health Social and Behavioral Interventions
[8]County of San Diego, Health and Human Services Agency
[9]University of California San Diego, Institute for Genomic Medicine
[10]University of California San Diego, Department of Obstetrics Gynecology and Reproductive Sciences
[11]University of California San Diego, Center for Computational Biology & Bioinformatics
[12]University of California San Diego, Department of Computer Science & Engineering
[13]University of California San Diego, Department of Electrical and Computer Engineering
[14]San Diego State University, Department of Electrical and Computer Engineering
[15]Micron Technology, Inc.

*Corresponding author: rfieldingmiller@health.ucsd.edu

This PDF file includes:

Materials and Methods

Other Supplementary Materials for this manuscript include the following:
Data S1: Wastewater and nasal-swab sequencing data GISAID accession details
SEARCH co-authors and affiliations

Methods

Human Subjects and IRB

All study procedures were reviewed and approved by the University of California, San Diego Institutional Review Board (IRB), which determined the human subjects portion of the study to be minimal risk and therefore exempt from oversight (protocol 201627). All participants provided informed consent (if 18 or above) or assent plus parental consent (if under the age of 18). Among participants who consented to diagnostic testing, median student age was 8.5 years (range: 0 – 17.5 years). Median staff age was 42.5 (range: 18 – 78). Among students, 37.6% were female, 39.8% were male, and 22.6% of parents declined to provide information related to gender. Among staff, 67.3% of consented individuals were female, 14.2% were male, and 18.6% declined to state. Approximately 23% of students and 34% of staff identified as white and non-Hispanic, 63% of students and 54% of staff identified as Hispanic, and 6% of students and 3% of staff identified as Black or African American.

Statistical analyses

Analysis was undertaken to identify the frequency with which the source generating a positive environmental sample could be identified. We assessed concordance between environmental samples and diagnostic testing by determining how often a positive diagnostic test preceded or coincided with a positive environmental sample (retrospective analysis) and conversely, how often a positive environmental sample coincided or was followed by a positive diagnostic test (prospective analysis). Under the retrospective analysis, the unit of analysis was positive diagnostic tests (n=89), and the retrospective outcome was the proportion of diagnostics tests preceded/coincided, within 7-days, by a positive wastewater or positive surface sample. Under the prospective analysis, the unit of analysis was positive wastewater samples (n=133) and positive surface samples (n=374), and the prospective outcome was the proportion of environmental samples coincided/followed, within 7-days, by a positive diagnostic test. We computed 95% confidence intervals for the retrospective and prospective outcomes by randomly sampling 10,000 times from independent normal distributions for the two proportions. Analysis was limited to sites that participated in the SASEA program throughout the validation phase and whose diagnostic test could be linked back to a classroom (for assessing concordance between diagnostic test and surface samples). All analysis were conducted in STATA version 16 (STATA Corp, College Station, Texas).

Sequencing

Libraries were pooled with equal volume and sequenced with Single Read 26 basepairs (SR26) on an Illumina MiSeq using a MiSeq Reagent Nano Kit V2 to determine volumes for balanced loading.

All sequencing data were analyzed with the COVID-19 VIral Epidemiology Workflow (C-VIEW). C-VIEW, available at https://github.com/ucsd-ccbb/C-VIEW, is an open-source, end-to-end workflow for viral epidemiology that is currently focused on SARS-CoV-2 lineage assignment and phylogenetics. tarting from raw sequencing data (.fastq) files, it performs alignment, variant identification, consensus sequence calling, lineage assignment, phylogenetic

tree building, and calculation of extensive quality control metrics. Additionally, it generates outputs suitable for public data deposition and incorporates metadata integration to support contact tracing. Cloud-based processing and storage are employed throughout C-VIEW to provide extreme scalability and robustness, and its parallel architecture is optimized for rapid time-to-result: processing an entire NovaSeq run takes approximately one hour.

C-VIEW is built on established open-source tools for viral amplicon-based sequencing*(1-3)*. Briefly, reads are aligned with Minimap2*(4)*, primer sequences are removed and quality filters are applied using iVar trim*(1)*, and variants are identified via samtools*(5)* mpileup and iVar variants*(1)*. Consensus sequences are then called with iVar consensus*(1)*, and a multiple sequence alignment is inferred using ViralMSA*(7)* wrapping around Minimap2*(4)*. Pangolin*(3)* is employed to derive SARS-Cov-2 lineages from consensus sequences, and phylogenetic trees are inferred using a maximum likelihood estimation by IQ-TREE*(2)*. Trees are visualized with Empress*(6)*.

1. Grubaugh, N. D. *et al.* An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol.* **20**, 8 (2019).
2. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).
3. Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* **5**, 1403–1407 (2020).
4. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018).
5. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
6. Cantrell, K. *et al.* EMPress Enables Tree-Guided, Interactive, and Exploratory Analyses of Multi-omic Data Sets. *mSystems* **6**, (2021).
7. Moshiri N. ViralMSA: massively scalable reference-guided multiple sequence alignment of viral genomes. *Bioinformatics* **37**, 714–716 (2021).

Data S1. (Separate file)

Wastewater and nasal-swab sequencing data GISAID accession details

SEARCH (San Diego Epidemiology and Research for Covid Health) Co-Authors:
Nathan A Baer[1],
Tom Barber[1],
Anelizze Castro-Martinez[1]
Marisol Chacón[1]
Willi Cheung[1,2,3]
Evelyn S Crescini[1]
Emily R Eisner[1]
Lizbeth Franco Vargas[1]
Charlotte Hobbs[4]
Alma L Lastrella[1]
Elijah S Lawrence[1]
Nathaniel L Matteson[5]
Karthik Gangavarapu5
Toan T Ngo[1]
Phoebe Seaver[1]
Elizabeth W Smoot[1]
Rebecca Tsai[1,6]
Bing Xia[1]
Stefan Aigner[1,2,7]
Catelyn Anderson[5]
Pedro Belda-Ferre[1,6]
Shashank Sathe[1,2,7]
Mark Zeller[5]
Kristian G Andersen[5]
Gene W Yeo[2,7]
Ezra Kurzban[5]

[1]Expedited COVID Identification Environment (EXCITE) Laboratory, Department of Pediatrics, University of California San Diego, La Jolla, CA
[2]Sanford Consortium of Regenerative Medicine, University of California San Diego, La Jolla, CA
[3]San Diego State University, San Diego, CA
[4]Rady Children's Institute for Genomic Medicine
[5]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA, USA
[6]Department of Pediatrics, University of California San Diego, La Jolla, CA
[7]Dept of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA