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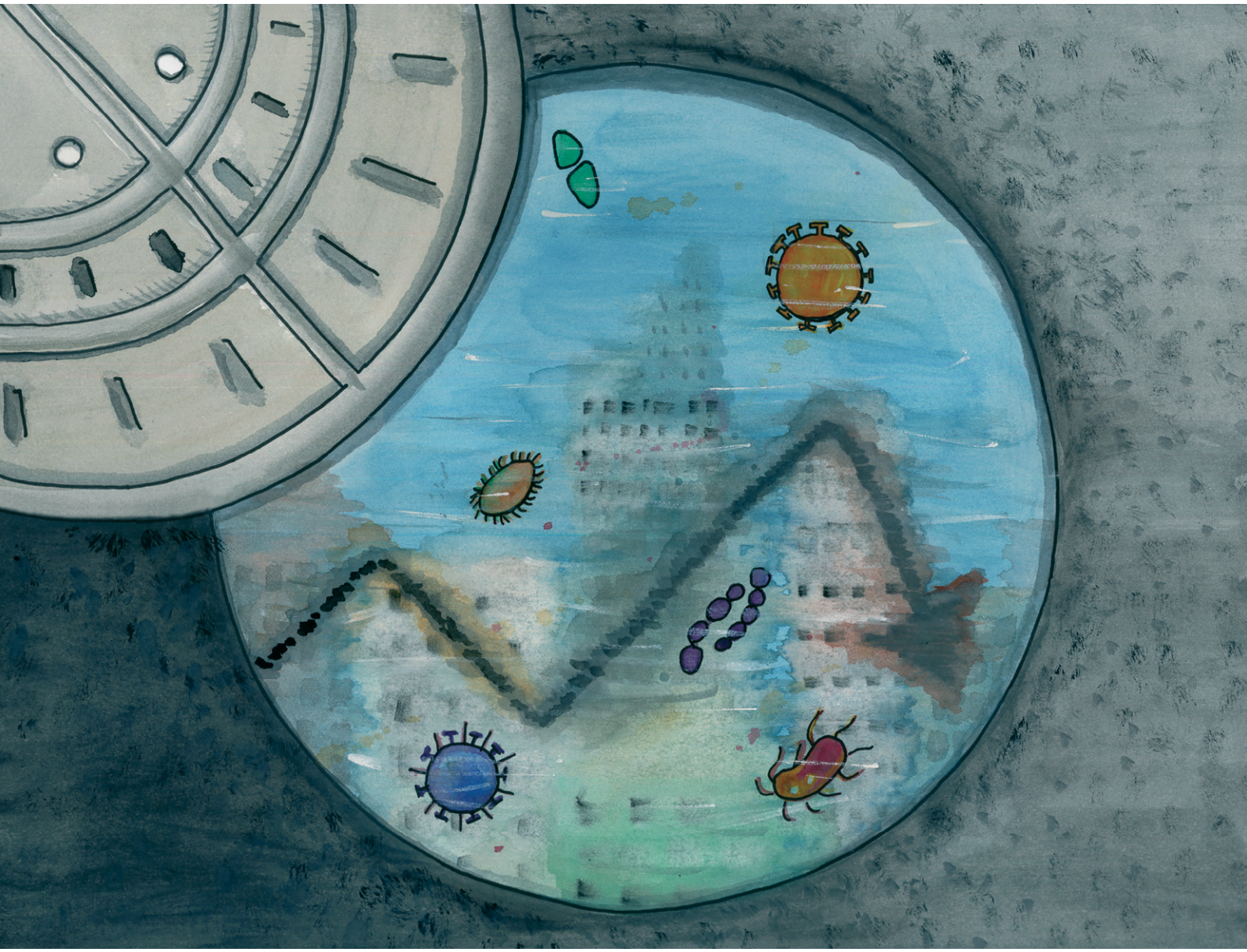

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Wastewater for public health: timely, sensitive, and reliable SARS-CoV-2 omicron variant monitoring in California

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The SARS-CoV-2 Omicron (BA.1/BA.1.1) variant was declared a variant of concern (VOC) on November 26, 2021 and rapidly became the dominant lineage globally. Monitoring for VOCs is a public health priority, but standard case-based surveillance requires a robust, local whole genome sequencing (WGS) network and results may take weeks. Wastewater monitoring of VOCs is appealing as a potentially sensitive and timely approach to VOC monitoring that could provide complementary information to case-based WGS and a more complete picture of VOC circulation. However, wastewater VOC monitoring through PCR assays and WGS each have unique advantages and disadvantages that are not well characterized. The California Department of Public Health collaborated with academic partners to conduct wastewater VOC monitoring during the emergence of Omicron BA.1./BA.1.1 in late 2021. Wastewater monitoring was conducted *via* RT-PCR assays targeting specific mutations and via wastewater sequencing. Wastewater data was analyzed within the context of case-based WGS data to track the emergence of Omicron in California. In most locations across California, wastewater PCR provided early identification of the emergence and subsequent dominance of Omicron BA.1./BA.1.1. These results were then corroborated and confirmed as case-based WGS results became available. These data helped guide real-time public health response during the Omicron surge. Wastewater monitoring of VOC's provides a timely, accurate, and sensitive methodology for VOC monitoring. Successful implementation of wastewater VOC monitoring requires wastewater surveillance infrastructure as well as ongoing investment and research to support the development and deployment of assays and sequencing methodologies for emerging variants.

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Water impact

COVID-19 variant monitoring provides valuable information, informing public health guidance, treatment recommendations, resource allocation, and personal risk assessment decisions. As clinical COVID-19 testing has decreased, alternative disease surveillance tools are increasingly important. This study demonstrates the reliability of wastewater monitoring to provide accurate and timely information about the spread of COVID-19 variants.

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1. Introduction

Detection of emerging variants of concern (VOCs) of SARS-CoV-2 (the virus causing Coronavirus disease 2019 [COVID-19]) and tracking of their circulation at the national, state, and local level (city and county jurisdictions) is an important public health priority. Health departments, healthcare providers, and the public may use information about variant circulation to inform risk assessments and decision making on COVID-19 prevention, resource allocation, and therapeutics. We describe the implementation and results of wastewater VOC monitoring in California during the initial

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recognition and rapid spread of the Omicron variant sublineage BA.1/BA.1.1 - the first strain of the Omicron VOC to become dominant (hereafter referred to as Omicron).

The Omicron variant was categorized as a VOC by the World Health Organization (WHO) on November 26, 2021, two days after the first case caused by that variant was reported from South Africa. Omicron was classified a VOC due to a high number of genomic mutations and the potential for higher transmissibility, severity, and ability to evade vaccines and existing therapeutics. Within a week of the WHO declaration, a case caused by the Omicron variant was identified in California, and the United States instituted a temporary travel ban from eight countries to slow importation of new cases. Omicron quickly became the predominant variant in the US and was associated with a surge of new cases. Omicron also attenuated the effectiveness of several first-line clinical therapeutics,[1,2] refocused the need for booster vaccinations, and led to re-implementation of nonpharmaceutical interventions such as masking mandates.[3–8]

Traditional surveillance for VOCs has primarily relied on whole genome sequencing (WGS) of isolates obtained from PCR-confirmed cases, and, when available, antigen positive tests. While surveillance based on WGS of case isolates (case-based WGS) provides important information, it is also resource- and time-intensive. In 2021, most states sequenced less than 5% of all polymerase chain reaction (PCR) confirmed case isolates and WGS results are typically not available until weeks after sample collection due to a variety of resource, logistic, and efficiency reasons.[9] While such levels of sequencing and result turnaround times likely allow for adequate routine VOC trend surveillance, it may not be adequate for timely detection of new, rapidly emerging VOCs such as Omicron. Monitoring VOCs through wastewater, a pooled community sample, can provide timely situational awareness that is representative of the entire community contributing to each wastewater treatment plant (*e.g.*, a city).[10,11] Monitoring VOCs at multiple wastewater treatment plants throughout a county or state can also provide a broader regional picture of VOC circulation. Wastewater monitoring in general has greatly expanded in recent years and is currently routinely used throughout the United States to track multiple seasonal pathogens (such as influenza and RSV) and emerging pathogens (such as mpox).[12] Many of these other viral pathogens feature important subtypes or variants marked by key genomic differences that could be monitored through wastewater in similar ways to the methods presented here for SARS-CoV-2 variants (*e.g.*, to monitor differing influenza subtypes, mpox clades, or norovirus genotypes). Because wastewater monitoring systems are already in place in many communities, and because VOC wastewater monitoring can be done through the testing of relatively few samples with results available within days, wastewater VOC monitoring is an attractive methodology to track the emergence and epidemiologic evolution of VOCs.

There are two complementary approaches to monitor VOCs in wastewater: targeted PCR assays either *via* digital droplet PCR [RT-ddPCR] or quantitative PCR [RT-qPCR] (hereafter referred to as wastewater PCR), and wastewater sequencing (through targeted amplicon sequencing or wastewater WGS). Wastewater PCR measures the concentration of VOC-specific characteristic mutations. Such assays are designed to be specific to each variant and can only be developed when at least part of the variant genome – derived from case-based WGS data – is already known. Wastewater PCR can provide results in as little as 3 hours (averaging 24–72 hours) from sample collection and provide quantitative estimates of VOC proportions for the entire community contributing to a sewershed.[11] While PCR assays for case-based samples can also have fast turnaround times, practical considerations often add to the time needed to estimate VOC proportions for a community. One reason for this increased turnaround time is that some laboratories using VOC assays may only choose to use them after first having a positive PCR test result for SARS-CoV-2. Another reason is that making estimates for an entire community based on individual case tests requires aggregation of multiple individual test results, each with their own delays in laboratory processing and submission, and additional time needed to aggregate data from multiple sources.

Sequencing of SARS-CoV-2 RNA in wastewater to identify VOCs, provides information on the relative abundance of different genomic sequences present within a sample. Sequence information can be compared against global databases of SARS-CoV-2 sequences to see if they match known mutation sequences from any number of variants or if they may represent novel mutation sequences. As such, estimates of all circulating VOCs in a community can be made from just a few wastewater samples. However, wastewater is a complex matrix including genomic fragments of multiple strains of SARS-CoV-2 (and thus likely multiple variants), and coverage of the genome captured within the wastewater sample can vary. Interpretation of sequences from fragmented genomes originating from multiple different SARS-CoV-2 lineages that have suffered degradation in wastewater can be challenging, with significant bioinformatic inference needed and a lower sensitivity to identify low-abundance lineages than PCR-based assays that specifically target a single lineage. Additionally, the time needed for a laboratory to perform WGS is similar to case isolate WGS. However, in our experience with California disease surveillance programs, practical turnaround times for wastewater sequencing data for surveillance can still be faster as compared to case-based WGS for surveillance. Most laboratories that perform clinical testing for the SARS-CoV-2 virus do not have WGS capacity; samples from these laboratories first must test positive for the SARS-CoV-2 virus before being shipped to another laboratory for WGS. And, since the results for multiple individual tests are needed before a VOC proportion estimate can be made for a community with accuracy, additional time is needed to wait for laboratory results to be submitted from multiple sources before then being aggregated. On the other hand, wastewater sequencing is generally done at the same laboratory performing

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wastewater extraction and PCR analysis, and far fewer tests are needed to develop community level proportion estimates.

The California Department of Public Health (CDPH), as part of the Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) and in collaboration with academic partners, has been monitoring wastewater in California for SARS-CoV-2 since late 2020. These efforts have included advancing methodologies for VOC monitoring in wastewater through both wastewater PCR and wastewater sequencing, two distinct modalities with different advantages and disadvantages in utility for public health surveillance. We describe here results of wastewater monitoring for Omicron in 20 sewersheds across California and compare the timeliness, sensitivity, and reliability of wastewater monitoring to that of the current public health gold-standard for determining VOC estimates, case-based WGS surveillance. We then share perspective on the utility of PCR and sequencing-based wastewater surveillance for the real-time Omicron public health response.

2. Methods

California surveillance of wastewaters network (Cal-SuWers network)

The Cal-SuWers network is a collaborative effort between CDPH, CDC, the State Water Resources Control Board, local health departments (LHDs), private laboratories, and academic partners. Wastewater PCR monitoring for Omicron was implemented at 20 sites administered by different academic partners: the Sewer Coronavirus Alert Network (SCAN [Stanford, Emory]), Healthy Central Valley Together (HCVT [University of California, Merced and University of California, Davis]), and COVID-Wastewater Epidemiology for the Bay Area (COVID-WEB [University of California, Berkeley]).

Wastewater monitoring: SCAN and HCVT

SCAN has been measuring daily concentrations of SARS-CoV-2 RNA in settled solids – collected from primary influent wastewater settling tanks – since November 2020. SCAN also developed and retrospectively tested variant-specific assays for multiple SARS-CoV-2 variants (including Alpha, Delta, Omicron, and others) and began prospectively monitoring wastewater settled solids for SARS-CoV-2 variants in April 2021. Healthy Central Valley Together (HCVT), a collaboration between the University of California, Merced and the University of California, Davis, has been monitoring wastewater at two Central Valley locations since October 2021. During the time of this study, all laboratory processing for HCVT sites was performed through SCAN. Twelve sites were monitored by SCAN and HCVT, collectively analyzing wastewater from about 4.3 million people. All laboratory methods have been described in detail elsewhere.[13–15] Additionally, a laboratory protocol is publicly available on protocols.io.[16]

SCAN has tracked characteristic mutations for VOCs, including Alpha, Mu, Gamma, Beta, Delta, and Omicron in wastewater using custom-designed, targeted, reverse transcriptase ddPCR (RT-ddPCR) assays.[13,14] The characteristic mutation that had previously been used to detect Alpha - a deletion in the S protein at amino acid positions 69/70 (delHV69-70) - is also present in Omicron BA.1/BA.1.1, and is *not* present in Delta. Thus, this delHV69-70 assay was re-deployed in all SCAN sewersheds on November 29, 2021 for use on all prospective samples and retrospectively on samples collected between November 24 and November 29 from Merced, Modesto, San Jose, and Palo Alto. Concurrently, an assay specific to Omicron BA.1/BA.1.1, targeting a characteristic mutation in the S protein at amino acid positions 143–145 (delVYY143-145), was developed and confirmed to be sensitive and specific for Omicron.[13] The del143-145 assay was deployed prospectively on all SCAN and HCVT sites on December 4, 2021 and retrospectively on all delHV69-70-positive samples between November 25 and December 4, 2021.[13,16] To confirm the accuracy of these two assays, targeted sequencing of a portion of the S gene was conducted on a wastewater sample positive for both targeted assays. The RNA template from this sample was amplified using ARTIC V4 73R and 74 L primers.[10] The resultant 186 base-pair amplicon was then sequenced.

Wastewater PCR-based Omicron proportions were calculated from RT-ddPCR assay results by dividing the concentration of the del143-145 mutation by the concentration of a conserved portion of the SARS-CoV-2 N gene (representing the approximate total concentration of SARS-CoV-2) in wastewater.[14]

Wastewater monitoring: COVID-WEB

The COVID-WEB (Wastewater Epidemiology for the Bay Area) project began measuring concentrations of a conserved portion of the SARS-CoV-2 N-gene (representing the approximate total concentration of SARS-CoV-2, including all variants) in 24-hour composite wastewater influent samples in the fall of 2020. Laboratory analysis of samples was performed at the University of California, Berkeley (UCB), and laboratory methods are described in detail in Kantor *et al.*, 2022 and on protocols.io.[17,18] Wastewater PCR assays for VOC monitoring utilized allele-specific reverse transcription qPCR (RT-qPCR). An assay for the Delta-specific T19R mutation was deployed for samples collected between October 17, 2021 and November 23, 2021 (assay details described in Lee *et al.*, 2021).[19] It was suspected that wastewater samples positive for the T19-wildtype (WT19), present in non-Delta variants, were likely to be Omicron, given known dominance of the Delta variant at the time. So, the WT19 assay was first deployed on October 22, 2021 on select samples and was then run on all samples from November 30, 2021 onward (assay described in Lee *et al.*, 2021).[19] Concurrently, development of two Omicron-specific RT-qPCR assays targeting S:S371L, S373P and S:Q493R, G496S, Q498R was underway at the CDPH Viral and Rickettsial Disease Laboratory (VRDL). Omicron was confirmed in two T19-wildtype positive wastewater samples

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using these VRDL assays. Subsequently, other archived RNA samples at UC Berkeley were assayed using another Omicron-specific RT-qPCR assay, described in Lee *et al.*, 2022, to confirm the presence of Omicron.[20]

COVID-WEB also conducted targeted amplicon sequencing on wastewater samples utilizing the sequencing protocol and primers that had been successfully used to track the emergence of the Alpha and Gamma variants.[21] An in-house bioinformatics pipeline was designed to process the sequencing data (publicly available at NCBI, accession number PRJNA881396). Reproducible code, and the analysis workflow are available at **https://github.com/rosekantor/wbe_sarscov2_sequencing**.

COVID case data and case-based WGS for variant detection

Sewershed shapefiles were provided by each of the Publicly Owned Treatment Works (POTW). The number of PCR-confirmed COVID-19 cases reported to CDPH as a function of episode date (earliest of reported symptom onset, laboratory result, or case record creation dates) residing within each sewershed was determined using methods reported previously.[14] COVID-19 case isolate variant data were generated by the network of California laboratories known as "California COVIDNet" (a major source of case-based genomic surveillance in California, composed of local public health laboratories, VRDL, academic partners, and commercial laboratories) and joined with case data *via* the state's reportable diseases system, CalREDIE.[22] Collection date was defined as when a case isolate sample was collected and result date as when the laboratory result was received by CDPH. Case isolate WGS results were assigned to each sewershed based on residential ZIP-code and episode date. The PANGO lineage was assigned based on the version available at the time of data extraction, with the most recent results using pangoLEARN and pango-designation v1.2.66 (**https://github.com/cov-lineages/pangoLEARN/releases/tag/2021-08-24**).[23–25] Case-based WGS estimates of VOC abundance were calculated by dividing the number of sequences identified as Omicron (using WHO definition and including all PANGO sublineages BA.1 and BA.1.1)[2,24,25] by the total number of isolates sequenced from individuals residing in the sewersheds.

Statistical analyses

Spearman correlations analyses were performed with sampling data from SCAN/HCVT sites between the wastewater and case isolate VOC abundance estimates, comparing the mean ratio of the variant-specific mutation measured in wastewater (del143-145 in the S gene/SARS-CoV-2 N gene) to the proportion of case isolates sequenced and characterized as BA.1 or BA.1.1 sub-lineages, each averaged over the previous 14 days. Correlations were also measured between case-based WGS VOC abundance estimates of BA.1/BA.1.1 and wastewater sequencing-estimated BA.1/BA.1.1 abundance estimates. Further analyses assessed correlations between concentrations of the T19-wildtype in wastewater and the wastewater sequencing-estimated BA.1/BA.1.1 abundance in wastewater. For this analysis, COVID-WEB utilized a RT-qPCR platform, and the standard curves for the assays targeting the SARS-CoV-2 N-gene (representing total SARS-CoV-2 concentrations) and the assays targeting Delta (T19R) and Omicron (WT19) variants were not calibrated against each other. Without this calibration step, there was concern that calculating VOC wastewater proportions using RT-qPCR data could result in invalid proportions. Thus, wastewater Omicron proportions were not calculated using the COVID-WEB wastewater PCR results.

Statistical significance was set at $p < 0.05$; analyses were performed in R studio Version 2022.02.2.

Sensitivity of case-based WGS and wastewater-based monitoring

The sensitivity of case-based WGS to detect Omicron variants in the clinical population of a sewershed, given the number of sequenced specimens per week, was calculated according to eqn (1).[26]

$$\Phi = 1 - (1 - p)^{1/N} \quad (1)$$

In eqn (1), Φ represents the maximum prevalence of a novel variant that would be detected, at least once during a given period, with a probability of p. N represents the number of sequenced cases in the sewershed per week. For this analysis, p was set at 95%.

To assess the sensitivity of wastewater-based VOC surveillance, comparisons were made against case-based VOC surveillance. For wastewater-based VOC surveillance, results from PCR assays were used because that was the primary method being routinely used at the time. For case-based VOC surveillance, results from WGS were used as that was the primary method being used at the time, and VOC-specific PCR testing of case isolates was not widely being done, and those results were not being collected and aggregated for surveillance purposes. While these do not allow a direct comparison of assay-based or WGS-based technologies, they allow a comparison of two different VOC-surveillance systems utilized by public health.

Sensitivity of wastewater PCR to detect Omicron was inferred by comparing the date of the first Omicron detection in wastewater with the date of the first confirmed case detected by case-based WGS. The variable sensitivity of case-based WGS to detect a case of Omicron (which is dependent on the number of specimens being sequenced) provided a gradient to compare against the sensitivity of wastewater PCR for detecting the introduction of Omicron to different geographic areas. If the first Omicron detection in wastewater preceded the first case-based WGS-confirmed case, then wastewater was considered more sensitive than case-based WGS in that geographic area. If the first Omicron detection in wastewater lagged the first case-based WGS-confirmed case, then wastewater was considered less sensitive than

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case-based WGS in that geographic area. Inter-region comparison of the time lags between the first detection of Omicron in wastewater *versus* case-based WGS quantified the relative performance of wastewater surveillance for early detection of Omicron in different regions of California, compared to case-based WGS surveillance.

3. Results

Validation of wastewater PCR for wastewater VOC detection

SCAN detected evidence of circulating Omicron variants *via* two wastewater PCR assays (delHV69-70 and del143-145). Targeted sequencing of a portion of the S-gene, performed on a positive sample (collected on December 2, 2021 from Sacramento, CA), confirmed the presence of the 214 EPE three amino acid insertion and the N211I single amino acid mutation, both characteristic of Omicron (BA.1 and BA1.1), which built confidence that both of SCAN's assays were effective at detecting Omicron.

COVID-WEB utilized a wastewater PCR assay for T19-wildtype (WT19) to detect the Omicron variant in wastewater samples. Two of these WT-19 positive samples were confirmed to be Omicron positive samples by the assays developed by VRDL, targeting the S:S371L, S373P and S:Q493R, G496S, Q498R mutations and the assay developed by Lee *et al.* 2022.[20]

Timeliness of initial detection of omicron presence

Mutations suggestive of BA.1 and BA.1.1 were identifiable in wastewater during a time of relatively low community transmission (21 to 90 cases per 100 000) and low overall wastewater SARS-CoV-2 concentrations (3300 to 46 000 copies per liter), as compared to the Delta peak wastewater concentrations of over 1.6 million copies per liter.

The first wastewater samples tested for Omicron were initially analyzed using assays that were not specifically developed to target BA.1/BA.1.1 (WT19 and delHV69-70) but were expected to accurately differentiate between Omicron BA.1/BA.1.1 and the Delta variant. After Omicron was declared a VOC with concern for introduction into California, COVID-WEB retrospectively tested a wastewater sample collected on November 22, 2021, which tested positive for WT19 on November 30. This was the earliest collection date for a wastewater sample that was presumptively positive for Omicron in a California sewershed. Collection dates of the first presumptive positive wastewater samples tested with the delHV69-70 assay were collected prospectively in Kern, Sacramento, Yolo, and Merced counties between November 25th and November 31st, with results available on December 1 and 2, 2021. These results were interpreted as highly suggestive of the presence of Omicron in wastewater and were communicated to local health jurisdictions as presumptive positives.

The specificity of these initial results was later confirmed on December 10, 2021 for WT19 positives and on December 2, 2021 for delHV69-70 positives with multiple Omicron-specific assays and targeted sequencing of a portion of the S-gene. The Omicron-specific del143-145 assay replaced the delHV69-70 assay for SCAN sites from December 4, 2021 onwards. Subsequent test results were considered positive for presence of Omicron.

By December 1, 2021, while assay validations were being concluded, presumptive positive wastewater detections of Omicron were included in CDPH situational updates, were being messaged to affected counties and were considered 'suggestive of Omicron circulation.' Sewershed-specific comparisons between first detections of Omicron in wastewater (including presumptive wastewater detections for those tested before December 4, 2021) *versus* first detections of Omicron in case isolates are presented in Table 1. These comparisons highlight the increased timeliness provided by wastewater monitoring for the initial detection of Omicron in multiple sewersheds in California. When comparing sample collection dates, the first wastewater samples positive for Omicron were collected a median of 3 days earlier than the first clinical isolates positive for Omicron (range: 21 days earlier to 9 days later). Comparing by result date, Omicron positive results were available by wastewater PCR a median of 10 days earlier than case-based WGS (range: 26 days earlier to 1 day later).

The column entitled 'Percent Variant Detectable by Case-based WGS' – which conveys the amount of case-based WGS capacity in each sewershed during this time period – demonstrates a pattern in the timeliness of the detection of Omicron *via* wastewater PCR *versus* case-based WGS. Timeliness of wastewater PCR (as measured by sample collection date) varied by the amount of case isolate WGS being performed in each area. In ten of the twelve sewersheds where the amount of case isolate sequencing was relatively lower (lower sensitivity and $\Phi > 1.5\%$), wastewater PCR detected Omicron earlier than case isolate WGS. The exceptions include Fresno and Calexico, where case isolate WGS detected Omicron two days earlier than wastewater (Table 1). In the eight sewersheds with more abundant case isolate sequencing (higher sensitivity and $\Phi \leq 1.5\%$), case isolate WGS detected the emergence of Omicron earlier than wastewater PCR in all except Sacramento and San Bernardino, where wastewater PCR detected Omicron first (Table 1).

Turnaround time for wastewater PCR, from sample collection to report date ranged from 1 to 8 days, with a median of 1 day. Between December 2021 and the time of this publication, this turnaround time had not changed substantially. Turnaround time to receive the case isolate WGS results for the first identified case of Omicron in each of the 15 counties in this study ranged from 3 to 23 days, with a median of 9 days (Table 1). Median case-based WGS turnaround time (all case isolates that underwent WGS) across the 15 counties included in this study in December 2021 was 14 days (20 days across California). This case-based WGS turnaround time steadily increased as the number of

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Table 1 Comparison of first detection dates of Omicron BA.1/BA.1.1 at sewersheds in California, presented in terms of sample collect date and sample result date for both wastewater (WW) PCR samples and case isolate WGS samples. For SCAN sites, wastewater samples tested before December 4 were tested with the delHV69-79 assay and samples tested after December 4 were tested with the del143-145 assay. For UCB sites, initial positive WT19 samples were confirmed with more specific assays by December 10. Percent of circulating variant detectable by case isolate WGS[a] was calculated for each sewershed catchment area in order to demonstrate how the abundance of local case isolate sequencing relates to the lead or lag time between first detections in wastewater and first detections in case isolate sequencing. Turnaround time is calculated as the number of days between the date of sample collection and the date each positive result was available (*i.e.* the time needed for sample collection, shipping and handling, laboratory analysis, and result reporting). WW PCR here refers to RT-ddPCR and RT-qPCR assays targeted to specific characteristic mutations of BA.1/BA.1.1, as described in methods

				First detection *via* WW PCR		First detection *via* case-based WGS		WW Lead time (date of first detection by case-based WGS - date of first detection by WW PCR)[b]		Turnaround time	
										WW PCR	Case-based WGS
County	Sewershed	Data source	Percent variant detectable by case-based WGS[a]	Collection date	Result date	Collection date	Result date	Lead time: collect date [days]	Lead time: result date [days]	Turnaround time [days]	Turnaround time [days]
Merced	Merced	SCAN/HCVT	4–6	11/25/2021	12/2/2021	12/16/2021	12/27/2021	21	25	7	11
Kern	City of Bakersfield	UCB	2–3	11/30/2021	12/1/2021	12/16/2021	12/27/2021	16	26	1	11
Marin	Las Gallinas	UCB	9–25	12/1/2021	12/3/2021	12/15/2021	12/18/2021	14	15	2	3
Sacramento	Sacramento	SCAN	0.8–1	11/30/2021	12/1/2021	12/9/2021	12/21/2021	9	20	1	12
Stanislaus	Modesto	SCAN/HCVT	3–4	12/10/2021	12/17/2021	12/18/2021	12/27/2021	8	10	7	9
Contra Costa	Delta diablo	UCB	1.5–1.8	12/7/2021	12/8/2021	12/15/2021	12/18/2021	8	10	1	3
San Francisco	Oceanside	SCAN	6–7	12/10/2021	12/11/2021	12/17/2021	1/3/2022	7	23	1	17
San Bernardino	San Bernardino	UCB	0.2–0.4	11/30/2021	12/1/2021	12/7/2021	12/14/2021	7	13	1	7
San Francisco	Southeast	UCB	6–7	11/22/2021	11/30/2021	11/28/2021	12/1/2021	6	1	8	3
Yolo	Davis	SCAN	7–10	12/13/2021	12/14/2021	12/17/2021	12/30/2021	4	16	1	13
Yolo	Sacramento	SCAN	7–10	11/30/2021	12/1/2021	12/2/2021	12/9/2021	2	8	1	7
San Mateo	Silicon valley	SCAN	6–7	12/15/2021	12/16/2021	12/16/2021	12/27/2021	1	11	1	11
Santa Clara	Gilroy	SCAN	0.4–0.6	12/13/2021	12/14/2021	12/12/2021	12/21/2021	-1	7	1	9
Alameda	East Bay municipal Utility District	UCB	1.5	12/1/2021	12/3/2021	11/30/2021	12/3/2021	-1	0	2	3
Fresno	Fresno	UCB	1.6–2	12/12/2021	12/14/2021	12/10/2021	12/19/2021	-2	5	2	9
Imperial	Calexico	UCB	8–13	12/15/2021	12/16/2021	12/13/2021	1/5/2022	-2	20	1	23
Los Angeles	LA city (Hyperion)	UCB	0.1–0.2	11/30/2021	12/3/2021	11/27/2021	12/2/2021	−3	−1	3	5
Santa Clara	San Jose	SCAN	0.4–0.6	12/11/2021	12/13/2021	12/6/2021	12/13/2021	−5	0	2	7
Santa Clara	Palo Alto	SCAN	0.4–0.6	12/7/2021	12/9/2021	12/1/2021	12/9/2021	−6	0	2	8
Santa Clara	Sunnyvale	SCAN	0.4–0.6	12/13/2021	12/14/2021	12/4/2021	12/13/2021	−9	−1	1	9

[a] Percent Variant Detectable by Case-based WGS: a metric used to describe the sensitivity of case isolate WGS in a sewershed or region. If a variant is circulating locally at the reported percent, case isolate WGS-based surveillance will have the capacity to detect this variant, within a week, with 95% confidence (see Methods). [b] To calculate lead time of WW PCR as compared to case-based WGS for detecting the introduction of Omicron BA.1/BA.1.1 into sewersheds, the date of the first wastewater detection was subtracted from the date of the first case-based WGS Omicron case in each sewershed. A positive value indicates that the variant was detected in wastewater first and a negative value indicates that it was detected *via* case-based WGS first. Lead time was calculated by both the date of sample collection for both wastewater and clinical samples ('Collection Date') and by the date results were reported for wastewater and clinical samples ('Result Date'), to account for turnaround time.

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COVID-19 cases increased, reaching a median of 26 days across the 15 counties included in this study by January 2022 (27 days across California). Since 2022, with decreased capacity for routine sequencing state-wide, turnaround time for case-based WGS has increased yet further, with a median of 35 days in the 15 counties in 2023 (30 days across California).

Omicron trends and correlations

VOC trend estimates were available from wastewater sooner than from case-based WGS. Wastewater results did not change over time, since wastewater estimates are derived from just a few samples, whereas case-based estimates rely on results for up to several thousand case isolates processed by multiple laboratories, with results becoming available to public health at variable times. Within each of the 20 sewersheds included in this study, the total number of positive case isolates that underwent WGS in any 14-day period ranged from 3 to 9751. Data from approximately 50% of these sequenced case isolates were available within 2.5–3 weeks. Due to these reporting lags, case-based Omicron abundance estimates changed over time, as individual case isolate results became available.

Because most case-based WGS data are not available until 2–3 weeks after case isolate sample collection dates, there is substantial missingness of data in real-time. For a rapidly emerging lineage such as Omicron, such missingness of data can make real-time case-based VOC estimates challenging. However, such estimates become complete at a later date when more results become available and are 'back-filled', aggregated based on sample collection date, and adjusted. To assess how early Omicron trends compared using wastewater-based and case-based methods, wastewater estimates were compared against both contemporaneous and back-filled case-based Omicron proportions. Contemporaneous case-based Omicron proportions, based on sample collection date, represent what is known to public health at any given time, impacted by any lag caused by laboratory processing or reporting delays. These estimated proportions were calculated using all data available to CDPH on any given week (between November 2021 and January 2022). Wastewater-based estimates were also compared to 'back-filled' case-based Omicron proportions (also using sample collection date). These 'back-filled' estimates were generated after February 2022, and represent a complete dataset after all case-based WGS data for each week was fully received, processed, and aggregated. Fig. 2A and B demonstrate the temporal relationships between wastewater-based Omicron estimates and contemporaneous case-based Omicron estimates (Fig. 2A) and between wastewater-based Omicron estimates and 'back-filled' case-based Omicron estimates (Fig. 2B). Of note, comparisons of wastewater-based Omicron estimates are not shown against Nowcast or other real-time model-based projections that are commonly used and available to public health (see Discussion).

Once all case-based WGS data was compiled, accounting for result turnaround time, correlations

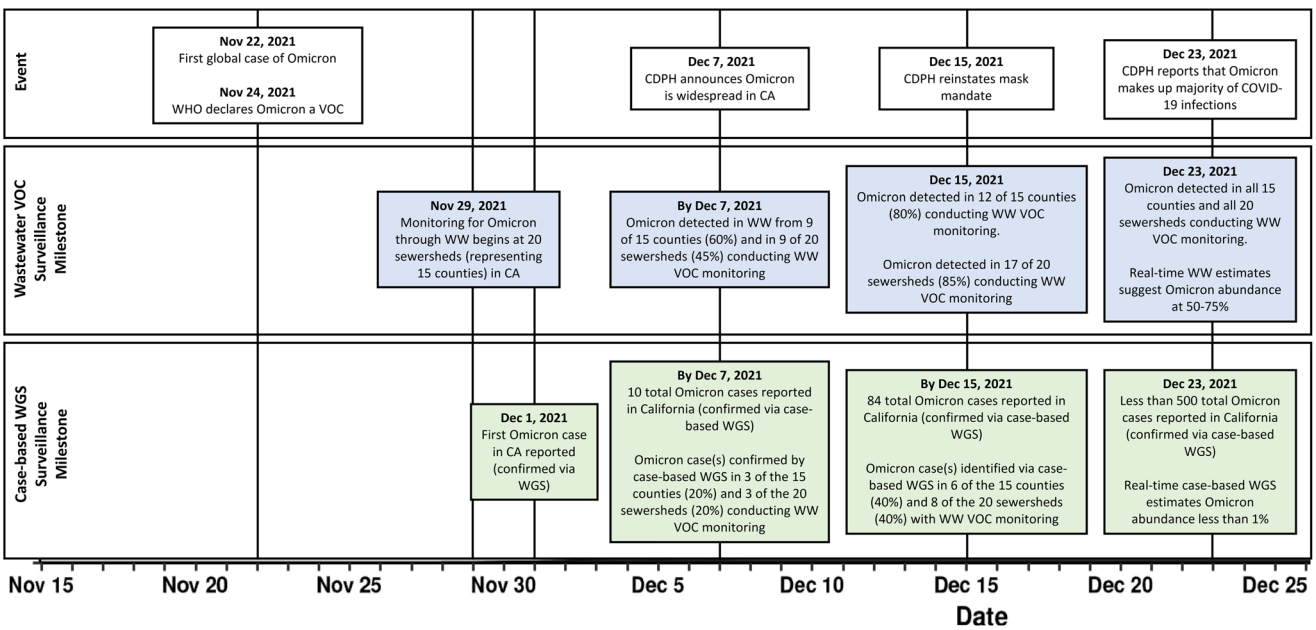

Fig. 1 Timeline of the omicron BA.1/BA.1.1 surge in California, with important time points plotted (white boxes represent the timing of notable events related to the omicron surge, blue boxes indicate wastewater-VOC monitoring-related milestones, green boxes indicate case-based WGS surveillance milestones). CDPH: California Department of Public Health, CA: California, VOC: variant of concern, WW: wastewater, WGS: whole genome sequencing.

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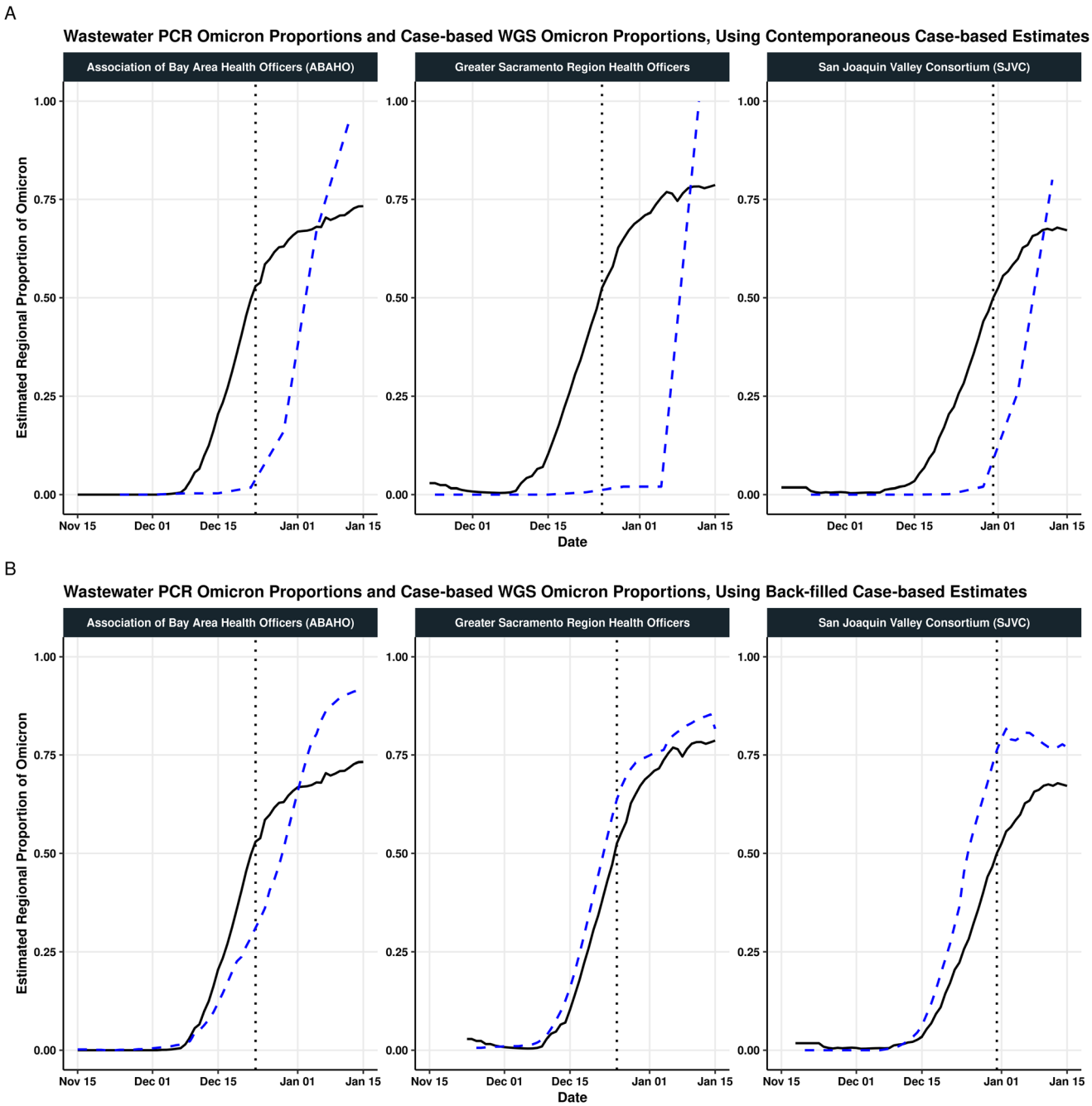

Fig. 2 Comparisons of surveillance estimates for Omicron abundance from wastewater PCR (using the Omicron-specific del143-145 assay [black solid lines]) and case-based WGS (blue dashed lines) between November 15, 2021 and January 15, 2022. The black vertical dotted lines represent the dates on which the regional proportions of Omicron crossed 50% in wastewater. **2A**: Wastewater PCR estimates of Omicron proportions and case-based WGS proportions, derived using the case data available and reported internally at CDPH at the time. By late December 2021, estimates of Omicron were at least 50% based on wastewater estimates and <10% based on contemporaneous case-based WGS estimates. **2B**: Wastewater PCR estimates of Omicron proportions and case-based WGS proportions, derived from all data available to CDPH as of the February of 2022, months after all sequencing data was reported. By late December 2021, estimates of Omicron were at least 50% based on both wastewater estimates and back-filled case-based WGS estimates.

between back-filled estimates of VOC proportions for case-based WGS and wastewater-based estimates were high. Trends in wastewater PCR Omicron abundance estimates follow closely and temporally the trends of case-based WGS Omicron abundance estimates during this period and were strongly correlated (Fig. 3). The mean Spearman's rank correlation coefficient among all sewersheds was 0.93 (SD: 0.08, range [0.76, 0.99]), and correlations were statistically significant ($p < 0.0005$) for all sewersheds analyzed (Fig. 3).

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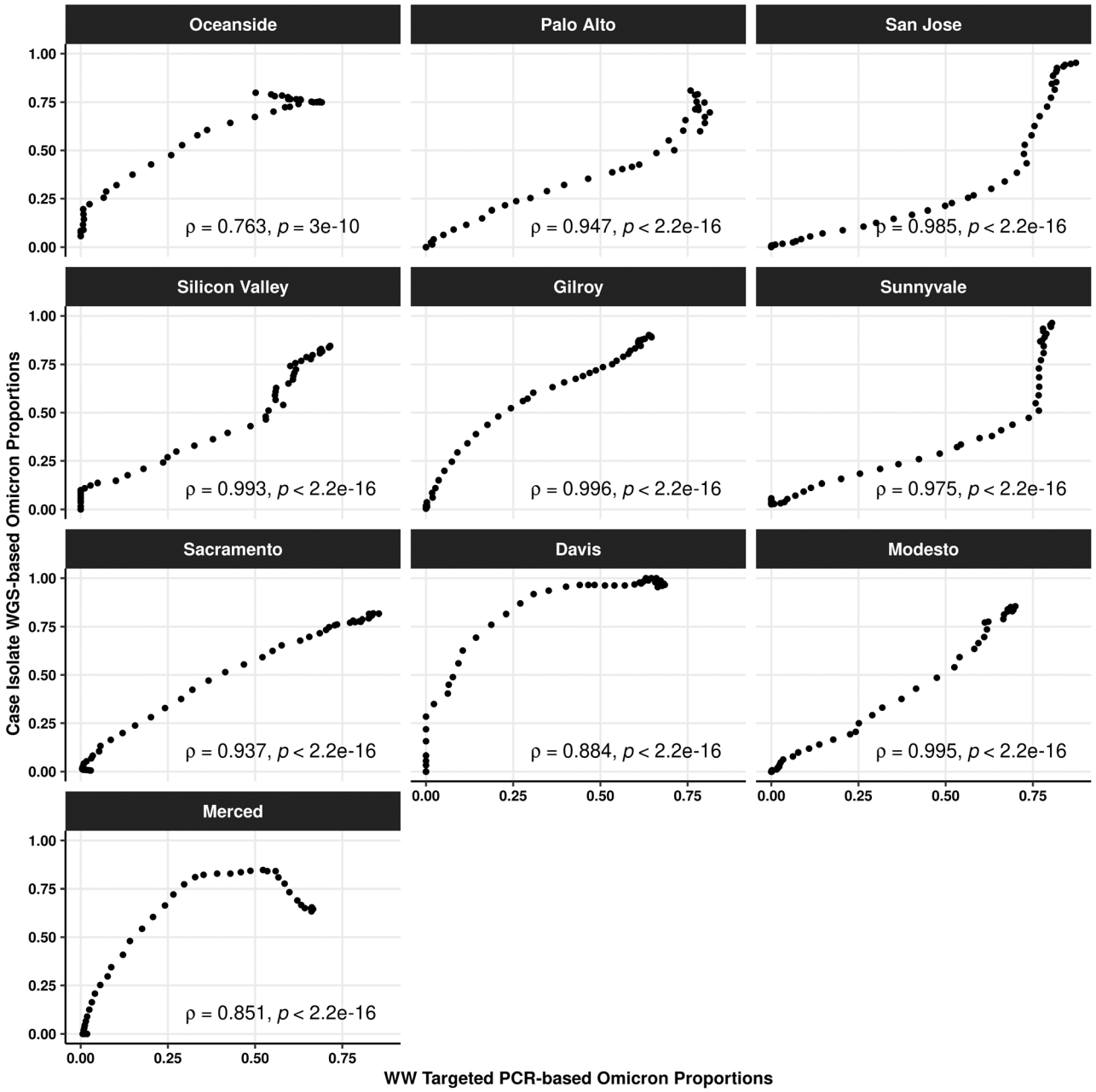

Fig. 3 Correlations between BA.1/BA1.1 estimates derived from wastewater PCR and case-based WGS, using 14-day rolling averages generated for each data set. Case-based WGS data were back-filled and inclusive of all data available after all cases had been recorded. Dots represent the 14-day rolling average data points and text represents results of Spearman correlations between case WGS-based proportions and WW PCR-based proportions.

Wastewater sequencing

At sites with wastewater sequencing available, sequencing results indicated that Omicron was circulating above 75% in all sewersheds by January 1 and that proportions of Delta had dropped to nearly zero by January 1, 2022 (Fig. 4). The mean Spearman's rank correlation coefficient between wastewater sequencing abundance estimates of BA.1/BA.1.1 and case-based WGS estimates of BA.1/BA.1.1 was 0.93 (SD: 0.08, range [0.8, 0.99]) among ten sewersheds with overlapping data. Correlations were statistically significant ($p < 0.05$) for all sewersheds except LA City (Hyperion). The mean Spearman's rank correlation coefficient between wastewater sequencing abundance estimates of BA.1/BA.1.1 and wastewater PCR-measured concentrations of the T19-wildtype (used by COVID-WEB, during this period, to infer wastewater concentrations of BA.1/BA.1.1) was 0.90 (SD: 0.11, range [0.70, 0.99]) among nine sewersheds with overlapping data. Correlations were statistically significant ($p < 0.05$) for Bakersfield, Calexico, Contra Costa (Concord), East Bay Municipal Utility District,

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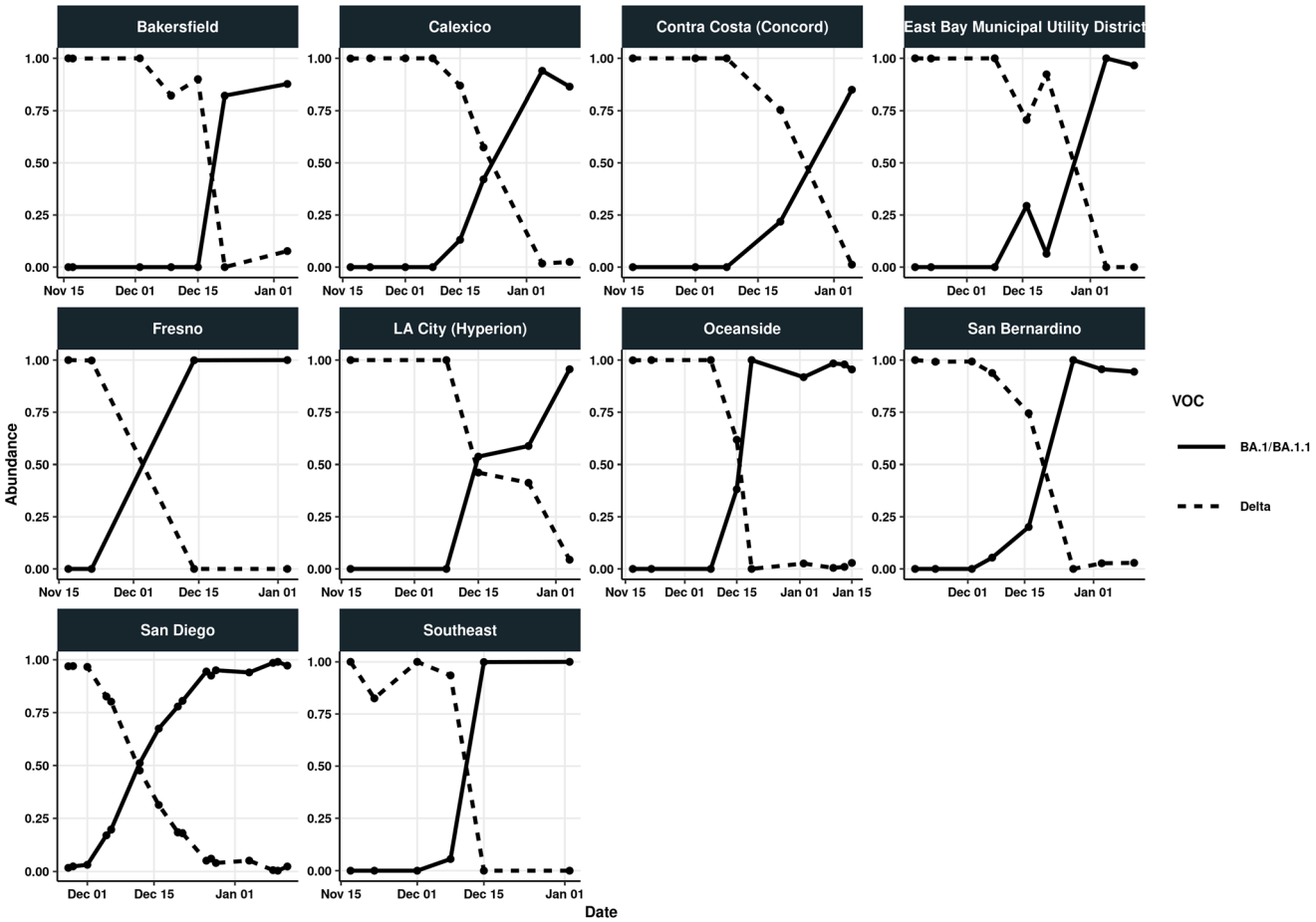

Fig. 4 Wastewater sequencing-estimated abundance of Omicron BA.1/BA.1.1 at sewersheds where wastewater-sequencing surveillance was active between November 15, 2021 and January 15, 2022.

and San Francisco Southeast. Although strong correlations were observed between wastewater sequencing and wastewater PCR abundance estimates and between wastewater sequencing and case-based WGS abundance estimates, sample sizes were low for all analyses (ranging from three to sixteen paired samples).

4. Discussion

Throughout the COVID-19 pandemic, monitoring of SARS-CoV-2 VOCs has been an important public health priority, and lessons learned regarding VOC monitoring can be useful to inform future public health situations when monitoring for COVID variants or for subtyping information of other viruses may be important. By building on existing wastewater surveillance infrastructure and experience using wastewater PCR assays and through wastewater sequencing, academic partners collaborated with public health to quickly monitor for the emergence of Omicron in California, at a time when the ability to monitor wastewater for VOCs through any means was just emerging as a possibility and wastewater sequencing was not well established. Wastewater VOC monitoring provided rapidly available, sensitive, and reliable data about the initial spread of Omicron in California. As case-based WGS results become available, those results subsequently built confidence in the wastewater-derived estimates. By providing important information about a new and rapidly spreading VOC, wastewater allowed for earlier state and local public health response. Importantly, wastewater surveillance is a complementary monitoring system: design of assays, confidence in, and interpretation of wastewater results was only possible through information generated by case-based WGS. However, as resources for case-based WGS shift, wastewater VOC monitoring can play an important role as a supplemental sentinel VOC surveillance method that is timely and accurate.

Early detection

Wastewater detections suggestive of Omicron were reported earlier than or essentially concurrently with case-based WGS surveillance. Notably, results include the earliest presumptive positive samples from each site, including some originally tested using non-specific assays in late November/early December 2021, prior to confirmation 1–2

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weeks later. These presumptive detections were included since the primary purpose of this analysis is to assess timeliness of wastewater PCR data availability compared to case-based WGS, and replacement with more specific assays would not have affected turnaround time. However, this experience highlights that the availability of wastewater results does not always immediately translate to actionable data. In assessing the timeliness of wastewater PCR for a newly discovered variant such as Omicron, additional time may be needed to build confidence in the interpretation of positive detections, whereas case-based WGS interpretation is more straightforward. In addition, PCR assays cannot be designed until a novel variant is sequenced, so wastewater PCR is not effective in detecting the emergence of an uncharacterized novel variant; however assay design and validation can be rapid once a variant is sequenced, as was done in this case for Omicron. Comparisons of the earliest detections of Omicron *via* wastewater sequencing were not able to be made as wastewater sequencing was not routinely or widely being done during the early emergence of Omicron in California.

During these first 1–2 weeks after Omicron was first declared a VOC, when wastewater Omicron monitoring was based on non-specific assays, the likelihood of the delHV69-70 mutation (previously an Alpha target) accurately identifying Omicron could be inferred given that case-based WGS surveillance had not identified Alpha cases in California for over 3 months. Similarly, with case-based WGS showing continued dominance of Delta (>99%) in CA and no known increasing lineages besides Omicron, increasing concentrations of WT19 in wastewater were highly suggestive of Omicron. Confidence in these assumptions was increased by subsequent confirmation with more specific assays (del143-145 for delHV69-70 positives and S371L, S373P, Q493R, G496S, and Q498R for WT19 positives) and wastewater sequencing (delHV69-70 positives). Thus, because the earliest deployed wastewater PCR assays for Omicron were non-specific, local variant information from regularly monitored case-based WGS were important to contextualize wastewater results. As such, even wastewater results prior to development of and confirmation by more specific assays and wastewater sequencing were considered presumptive positives; these were interpreted as, and messaged to impacted local public health as, highly suggestive of Omicron circulation.

Lead time for wastewater was partially driven by a faster turnaround time between collection and PCR result reporting, as compared to the turnaround time of case-based WGS (Table 1). The lead time in case-based WGS result reporting to public health has likely increased since December 2021. During that time, the median turnaround time for wastewater PCR was 1 day, which has not changed since. Case-based WGS capacity was high early in December 2021, when the overall number of COVID-19 cases were low and routine sequencing capacity was high. Since 2022, routine sequencing capacity across California has decreased, and median turnaround time for case-based WGS has more than doubled (median of 14 days in December 2021, *versus* 30 days in 2023 across California).

Comparing wastewater and case isolate sample collection dates, and thus excluding time-to-analysis, wastewater PCR detected Omicron before case-based WGS in eight out of 20 sewersheds. Looking closer at the lead time differences between sites, wastewater PCR outperformed case-based WGS surveillance for first detection primarily in areas where case isolate sequencing rates were relatively lower, whereas performance between wastewater and case-based WGS was similar when case-based WGS was robust and frequent (Table 1). Most of the sites where case-based WGS detected Omicron earlier than wastewater (excluding time-to-analysis) were from Santa Clara county, where case-based WGS frequency was very high (40% of all case isolates were sequenced at the time). Notably, the volume of case-based WGS (and thus sensitivity) has decreased substantially since 2022, including in the areas that previously had a high volume of case-based WGS.

Local VOC epidemiology

Wastewater PCR provided a 2–3 week lead estimate of the relative proportion of Omicron's circulation in local contexts, as compared to estimates derived from case-based WGS. Once specific wastewater PCR assays were developed and presumptive wastewater positives confirmed, wastewater monitoring VOC estimates were added to reports already being provided about where Omicron was being identified in wastewater, and provided as internal situational updates for public health leadership. For a rapidly changing VOC such as Omicron, a short lead window can be important for understanding a VOC's epidemiologic trajectory. By December 23, 2021, wastewater estimates suggested that Omicron made up 50–75% of all new infections in California, and CDPH reported that Omicron made up the majority of all CA COVID-19 infections (Fig. 1).[27] At that time, less than 500 total clinical cases in California (less than 1% of reported case sequences) had been confirmed as Omicron. These wastewater-based estimates were supported by CDC's Nowcast (a model-based projection), which also predicted a steeply rising dominance of Omicron, though lower than wastewater (Nowcast: ~23%).[9,28] Once all case-based WGS data was compiled, retrospective estimates of VOC proportions between case-based WGS and wastewater-based estimates were aligned, and correlations between the two surveillance metrics were high (Fig. 2B), supporting that the wastewater data were reliable.

Notably, given the known issues around case-based WGS representativeness as well as laboratory and reporting delays, model-based projections such as the CDC Nowcast are often used to inform public health situational awareness, rather than relying only on case-based WGS. However, the underlying data informing such models are case-based WGS

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data, and are thus still limited by the timeliness and robustness of that underlying data. Illustrating this point, the CDC notes that "projections for an emerging lineage with a high growth rate may have a higher degree of uncertainty (wider predictive interval) when it is just beginning to spread and still has low weighted estimates." Comparisons of wastewater VOC were not made directly against modeled estimates (such as the CDC nowcast) because the results of such estimates were fluctuating, and in general, the goal of this study was not to evaluate the performance of a model (which is dependent on each model's parameters and inputs). Additionally, during the first few weeks of the emergence of the Omicron VOC in California when overall case-based WGS confirmed cases were few, focus had been less on Omicron VOC proportional estimates and more on identifying when a county had identified a case and how many total counties had any cases.

Wastewater sequencing and wastewater PCR assays

Wastewater PCR and wastewater sequencing fill complementary roles in variant monitoring. Wastewater PCR results are available more rapidly than wastewater sequencing, which took 1–3 weeks during our study period. However, wastewater PCR assays can only be developed after variant sequences become available. Also, since mutations may be shared by multiple variants, PCR assays may not be specific to a single variant. Thus, assay development and result interpretation must be done within the local COVID genomic epidemiologic context, as provided by case-based WGS.

While wastewater PCR assays target one or a few pre-selected characteristic mutations, variant genomes can differ and do not all contain the same characteristic mutations. As such, wastewater PCR may underestimate the true proportion of a given variant. For example, in early January 2022, wastewater PCR estimates of Omicron abundance plateaued at around 75% in most sites. Estimates of Delta, the only other prominent variant thought to be circulating, remained low (<1%). This raised the specter of a new circulating variant not yet manifested in case-based WGS. Wastewater sequencing data, however, estimated that Omicron prevalence was over 95% (Fig. 4). Investigation of local case-based WGS data revealed that BA.1.1 was predominant over BA.1 across California and that some genomes of the BA.1.1 variant retained 143–145 residues. In fact, the local prevalence of the del143-145 mutation in BA.1.1 was estimated to be 70%, which was lower than the global estimates for prevalence of this mutation in BA.1.1 (92.2%).[27] So, although the abundance of the del143-145 mutation plateaued at 75%, case-based WGS and wastewater sequencing helped confirm that the overall abundance of Omicron BA.1/BA.1.1 was near 100% in California. Without the additional resolution provided by wastewater sequencing and case-based WGS, the wastewater PCR results would have been difficult to interpret.

Wastewater sequencing is moderately VOC-agnostic and does not require a previously known sequence. Bioinformatic processing of the presence, absence, and abundance of multiple mutations simultaneously can be used to infer information about multiple known VOCs and other emerging variants. As highly similar variant sub-lineages have become more dominant since the first emergence of Omicron, wastewater PCR assays are not always capable of distinguishing between all variants and subvariants. If a variant's growth advantage is not as high as Omicron BA.1/BA.1.1, the 1–3 weeks required for wastewater sequencing results may not always be as important for public health action or situational awareness. In these situations, wastewater sequencing may play a primary role for routine wastewater variant monitoring, augmented with targeted PCR assays to monitor for new or emerging variants as possible and depending on situational need.

Public health use

As with many new SARS-CoV-2 variants when they are first described, the public health risk of Omicron to California was unknown when it began circulating in the state. Omicron was associated with many worrisome features. There was evidence of immune escape and increased transmissibility (including after vaccination) from laboratory and epidemiologic studies from settings outside of California, potential increased clinical severity, and decreased therapeutic effectiveness. Understanding if these features indicated enough of a public health risk to warrant public health intervention (*e.g.*, messaging, masking guidance, change in therapeutics) required additional situational awareness, including signs of rapidly increasing local COVID-19 transmission, the rapidly increasing proportion of Omicron cases in California, and assessments of severity (*e.g.*, hospitalizations) in setting of local considerations (*e.g.*, hospital bed capacity).

By providing data about the introduction and spread of Omicron in California earlier than case-based WGS, wastewater VOC monitoring was directly beneficial for local and state public health awareness and action. Wastewater detections of Omicron in multiple sewersheds, within days of the first California case being identified, led to reporting on December 7, 2021 that Omicron was present across the state (Fig. 1).[29–32] At that time, there had only been ten reported cases in the state, while evidence of Omicron had been detected in wastewater from almost 50% of monitored sewersheds in 14 counties. Within the next week, several LHDs announced the presence of Omicron in their communities, as suggested by wastewater.[29–34] Subsequently, as increasing concentrations of Omicron (and of overall SARS-CoV-2) in wastewater suggested rising cases, at least two LHDs used wastewater results to help urge residents to get vaccinations.[35] In at least one LHD, wastewater detections prompted notification of local hospitals for surge capacity building and resource planning, ramped up community testing, and refreshed county public health pushes for vaccinations.[36] Wastewater data also helped inform some LHDs and hospital groups in decision making to stop using

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therapeutics rendered ineffective by the Omicron variant (*e.g.*, monoclonal antibodies).

Notably, in other instances, new variants have been associated with worrisome features warranting close public health monitoring, but local situational context has not ultimately required direct public health action. Whether or not action is needed, vigilant public health surveillance (including wastewater, case-based, and hospitalizations) is an essential first step in determining whether direct public health action is warranted.

Planning

As overall resources and interest in case-based sequencing for COVID-19 decline, wastewater monitoring has the potential to become more useful for public health monitoring of new and circulating variants, or even for adaptation for monitoring of other viral subtypes that would otherwise require WGS to characterize. Such a system can be strategically deployed to gain insight in areas that are of particular importance for situational awareness, particularly in regions where case-based WGS is difficult to maintain at robust levels.

For wastewater VOC monitoring to produce timely results, several elements need to be in place: existing wastewater monitoring programs, assays ready to deploy, the research and development capability to quickly develop new, sensitive assays, and laboratory reagent availability. Weeks are needed to establish wastewater monitoring at a new site, but adding VOC monitoring at an established monitoring site requires no additional sample collection. Within academic, public health, and commercial laboratories, resources are needed to design and validate wastewater PCR assays for new variants and to conduct wastewater sequencing.[14] Coordination at the national and international level is important to facilitate knowledge sharing about new variants and assay development. Coordination and communication between national and international organizations, academic and research organizations, and health departments will aid in timeliness and operationalization of wastewater monitoring for VOCs.

Conclusion

Wastewater monitoring, alongside case-based WGS, allowed California and multiple local health departments within California to track Omicron during a time of rapid change and uncertainty. This experience highlights the value of wastewater surveillance. As testing utilization and case-based WGS for COVID-19 declines, wastewater monitoring can provide a timely, sensitive, and reliable method for public health to monitor the emergence and circulation of viral variants.

Data availability

Data for this article, including wastewater variant proportions measured by SCAN and HCVT, are available publicly at the Stanford Digital Repository (**https://doi.org/10.25740/cx529np1130**). Data collected from clinical case isolates described in Fig. 2 and 3 are not available for confidentiality reasons.

Author contributions

Alexander T. Yu – Conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing – original draft, writing – review & editing. Elisabeth Burnor – Formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. Marlene Wolfe – Data curation, methodology, investigation, resources, funding acquisition, supervision. Rose Kantor - Data curation, methodology, investigation, resources, funding acquisition. Tomas Leon – Formal analysis, software, supervision. Sindhu Ravuri – Formal analysis, software. Madhura Rane- Writing – review & editing. Mayuri Panditrao – Formal analysis, methodology, writing – review & editing. Colleen Naughton - Data curation, methodology, investigation, funding acquisition, supervision. Angela Rabe – Investigation, writing – review & editing. Sasha Harris-Lovett – Investigation. Andrew Abram - Investigation, writing – review & editing. Deva Borthwick – Investigation. Bradley White – Investigation, methodology. Joaquin Bradley Silva – Investigation. Christina Lang – Investigation. Will Probert – Formal analysis, investigation. Deb Wadford - Formal analysis, investigation, supervision. Heather Bischel - Data curation, methodology, investigation, funding acquisition, supervision. Seema Jain – Supervision, writing – review & editing. Kara Nelson - Data curation, methodology, investigation, resources, funding acquisition, supervision. Alexandria B. Boehm - Data curation, methodology, investigation, resources, funding acquisition, supervision. Duc J. Vugia – Conceptualization, supervision, writing – review & editing.

Conflicts of interest

B. W. is an employee of Verily Life Sciences, LLC.

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