Analysis of the effectiveness of a pilot program to monitor SARS-CoV-2 presence in wastewater in New York City

Department of Environmental Protection Department of Health and Mental Hygiene February 17, 2022

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This report describes the New York City Department of Environmental Protection's (DEP's) pilot program to test New York City wastewater for SARS-CoV-2, the virus that causes COVID-19, as mandated by Local Law 28 of 2021 (LL28). The law calls upon DEP, in consultation with the New York City Department of Health and Mental Hygiene (DOHMH) to establish a program to quantify levels of SARS-Cov-2 in the influent wastewater stream at each New York City Wastewater Resource Recovery Facility (WRRF).

The purpose of this program was to evaluate the usefulness of wastewater-based epidemiology (WBE) in aiding the COVID-19 pandemic response. WBE is the analysis of pollutants, viruses and biomarkers in wastewater to obtain qualitative or quantitative data on disease transmission among inhabitants within a given sewershed. There is no evidence that the SARS-CoV-2 virus remains infectious in wastewater.¹ However, SARS-CoV-2 RNA (genetic material), can still be detected, which makes WBE for SARS-CoV-2 presence possible.¹¹

LL28 has eight reporting requirements, which are detailed in the sections below. Section 1 provides a brief summary of the reporting requirements, Section 2 provides the project timeline and sewershed map, and Section 3 provides a detailed explanation of selected reporting requirements.

1. Summary of responses to Local Law 28 reporting requirements

1.1 Results of sampling, disaggregated by the site where the sample was collected, date sample was collected, and date sample was tested, in order to monitor the leading indicators of increases or decreases in COVID-19 presence in each drainage area throughout the study

DEP initiated weekly measurements of SAR-CoV-2 RNA levels using reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) in wastewater in August 2020. Accompanying this report is a dataset containing results of sampling, disaggregated by the Wastewater Resource Recovery Facility (WRRF) sampling site, indicating dates of sample collection and testing, as well as quantity measurements of the detected SARS-COV-2 RNA. Trends in SARS-CoV-2 RNA levels measured in wastewaters correlated with trends inCOVID-19 cases reported to DOHMH. However, the science and techniques currently available are not yet fully robust enough to utilize levels of SARS-Cov-2 in wastewater as a leading indicator to predict present levels, or future trends in COVID-19 cases. It should be noted that NYC currently has relatively high COVID-19 diagnostic testing capacity, a robust COVID-19 surveillance system with mandated electronic reporting from laboratories to DOHMH, and the capacity to conduct extensive analytics to rapidly monitor, investigate and understand COVID-19 trends.^{III}

1.2 Cost of Pilot Program

WBE at DEP was built de novo at its Newtown Creek laboratory. Operating costs for the program totaled over \$520K for the 21-month period (April 2020 – December 2021), which translated to almost \$300K annually.

1.3 Analysis of the effectiveness of the pilot program in testing for SARS-CoV-2

The pilot was highly effective in developing NYC's capacity for WBE. It allowed DEP to establish methods to measure SARS-CoV-2 levels, detect SARS-CoV-2 variants in wastewater, and to develop strong relationships with academic, state, and federal partners.

1.4 Recommendations to expand the pilot program to include sampling at manhole sites and pumping stations if wastewater-based epidemiology detects SARS-CoV-2 in an amount, as determined by the commissioner of health and mental hygiene, that indicates a localized concentration of COVID-19^{iv}

As part of program development, DEP carried out localized monitoring in two NYC sewersheds to establish protocols. This effort demonstrated that subsewershed monitoring could be a useful method to localize

measurements of SARS-CoV-2 RNA. For DEP, the localized sampling effort was highly labor intensive and diverted staff from pollution prevention and monitoring duties required for DEP's regulatory compliance. Given the resources required to conduct subsewershed monitoring, without additional dedicated resources it is only feasible in very limited use, over small areas, when DOHMH determines a localized measurement is needed. Use cases will need to be developed by conducting further work to understand how to best interpret and utilize these localized measurements. At this time, DEP and DOHMH cannot recommend pursuing a localized sampling approach.

1.5 Recommendations to extend the pilot program for up to an additional six months if more testing is necessary, as determined by the commissioner, in consultation with the commissioner of health and mental hygiene^v

DEP and DOHMH recommend extending the pilot program for an additional year. Starting in January 2022, DEP will participate in a Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) program to test wastewater throughout the country for SARS-CoV-2. This program will use a third-party laboratory, LuminUltra, to perform testing. The program will run for one year and will be paid for by CDC. For an additional three months, DEP will conduct parallel testing using RT-qPCR. This testing will help DEP gain additional insight into the performance of its analytical methods.

1.6 A plan for weekly testing at each city wastewater treatment plant if the commissioner of health and mental hygiene or state commissioner of health declares that the incidence of SARS-CoV-2 is appropriate for such action or if the centers for disease control and prevention issues a SARS-CoV-2 pandemic declaration^{vi}

DEP expects its WBE partnership with DOHMH to continue as NYC plans for current and future public health emergency responses. As needs arise, and given adequate funding and staffing resources, DEP will be able to respond with monitoring of SARS-CoV-2 as well as other pathogens in the wastewater (e.g., flu, norovirus). At present, DOHMH finds information on SARS-CoV-2 levels and variants in wastewater useful for situational awareness. DOHMH concurs with the CDC and other researchers in this rapidly developing field that use of SARS-CoV-2 data from wastewater to estimate the number of SARS-CoV-2 infections is not recommended, due to uncertainties related to quantitatively comparing wastewater and clinical testing data. Expanded use of WBE data for more quantitative estimates of case rates is the focus of continued research nationwide, and DEP and DOHMH will build on their strong network of collaborators to stay abreast of new developments.

1.7 Recommendations to use a sequencing testing method other than PCR using N1 Primer to test samples, if the commissioner determines that such additional testing method is beneficial

DEP recommends the use of a targeted sequencing method to detect SARS-CoV-2 variants in wastewater. Through its partnerships with academic researchers, DEP utilized targeted sequencing to identify mutations on the spike protein of the SARS-CoV-2 virus. This sequencing approach proved effective in detecting several SARS-CoV-2 variants including Alpha, Delta, and Omicron in NYC's wastewater and is consistent with methods used by practitioners and researchers nationally and internationally.

1.8 Recommendations for making the pilot program permanent.

Through this pilot, DEP has mobilized operational and fiscal resources to respond to the COVID-19 pandemic, providing data to and working with DOHMH and other local and federal agencies. With the enrollment of DEP in the CDC NWSS program, the pilot program will continue for one year. At this time, the consensus within this emerging field is that there is a need for further development of the fundamentals of this technology. As knowledge in this emerging field of public health continues to be advanced, methods will continue to be improved, and additional applications of data from this program may be identified. DEP is committed to maintaining its engagement in this sphere through targeted collaborations with other water utilities, the NYC DOHMH, the academic community, and Federal entities to continue to develop the field of WBE so that it can be leveraged further in future public health emergencies.

2. Program timeline and map of sewersheds

The pilot program was researched, designed, and set up between April and July 2020. Starting in August 2020, DEP began reporting results to DOHMH. Between April 2020 and December 2021, DEP tested over 1,500 samples to monitor the amount of SARS-CoV-2 genetic material shed by populations served.^{vii} Major project and legislative milestones are shown in Figure 2.1.

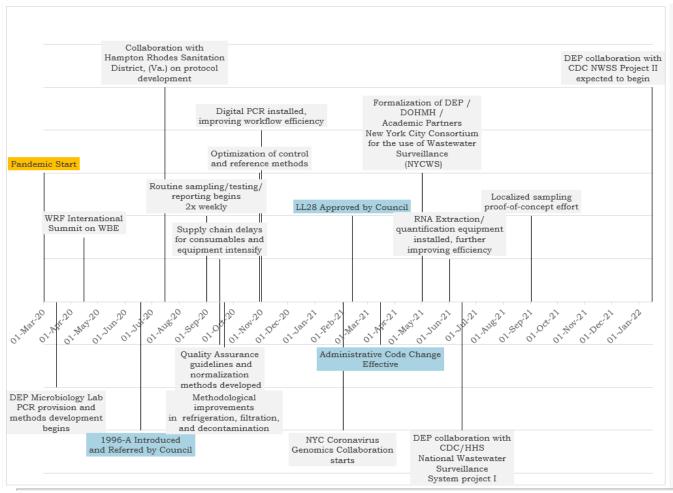
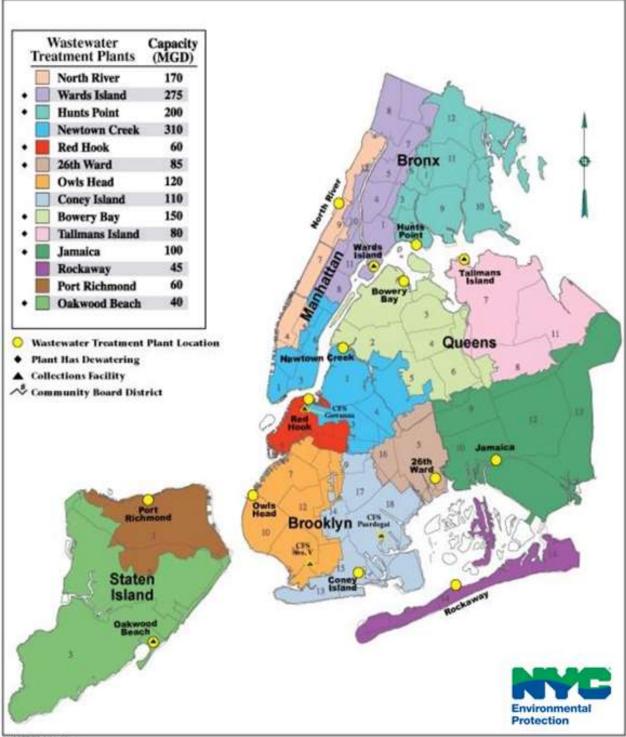


Figure 2.1. Milestones in pilot program development and implementation 2020-2021.

NYC's 14 WRRFs treat 1.3 billion gallons of wastewater daily. As shown in Figure 2.2, each WRRF serves a sewershed that covers residential, commercial and storm drain sources within one of fourteen geographic areas of NYC. For the pilot program, DEP collected samples of wastewater entering each of NYC's 14 WRRFs. The samples used for testing were 24-hour composites, i.e. sampling took place every three hours over a 24-hour period and the resulting samples were then combined into one sample corresponding to a 24-hour period. Details on methods used for testing are provided in Appendix 5.



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Figure 2.2. Map showing sewersheds served by NYC's 14 wastewater resource recovery facilities (WRRFs).

3. Detailed summary of responses to Local Law 28 reporting requirements

3.1. Results of sampling, disaggregated by the site where the sample was collected, date sample was collected, and date sample was tested, in order to monitor the leading indicators of increases or decreases in COVID-19 presence in each drainage area throughout the study

The levels of SARS-Co-2 RNA over time by NYC sewershed are provided in **Appendix 1 (submitted as a datafile).** Trends in SARS-CoV-2 RNA levels measured in wastewaters correlated with trends in COVID-19 cases in NYC (Figure 3.1). Evident in this data are matching trends during several waves of very high COVID-19 transmission, i.e. winter 2020-2021, summer 2021 and winter 2021-2022.

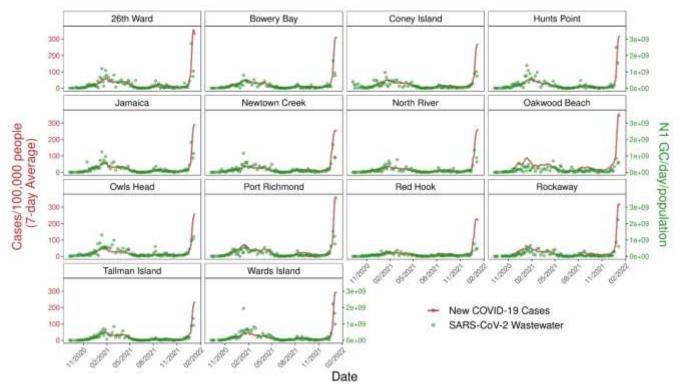


Figure 3.1. Summary of SARS-CoV-2 wastewater data for New York City's 14 sewersheds from September 2020 to January 2022. Right y-axis, green circles: SARS-CoV-2 viral loads in influent wastewater normalized by sewershed populations. Left y-axis, red line: 7-day average of new COVID-19 cases/day/100,000 people in the previous 7 days.

To evaluate how trends in SARS-CoV-2 levels can be used as a leading indicator, DOHMH performed a series of quantitative analyses to evaluate the temporal relationship between the levels of SARS-CoV-2 RNA in wastewater and COVID-19 disease surveillance indicators. This effort examined data from the 14 NYC sewersheds for the period September 2020 through May 2021 (i.e., from the beginning to the end of the second wave). Disease data included all SARS-CoV-2 PCR and antigen testing results by day

of test for NYC residents who were reported to the NYC DOHMH. Patient residential address from the test result was geocoded to residential sewershed to calculate the daily test percent positivity by sewershed. Percent positivity was used instead of case counts or case rates because the latter metrics are biased by healthcare seeking behavior. Artificially lower case rates are likely to be in the city areas where the population might be less able to seek testing because of financial, time, or access issues..

DOHMH used two methods to quantify the relationship between wastewater and disease data for the period of September 2020 through May 2021. Both methods considered the three phases of the second wave: (1) increasing phase (8/31/20-11/30/20); (2) peaking phase (12/1/20-2/28/21); and (3) decreasing phase (3/1/21-5/31/21). The first method fitted smoothed time-series for both indicators using natural cubic splines allowing 10 degrees of freedom for the entire study period, and then identified the inflection (i.e., where the slope gets steeper), peak, and secondary peak (or shoulder) dates that respectively fall in these three phases to determine the number of leading or lagging days in each of the 14 sewersheds. Paired t-tests were then applied to the lead/lag days across the 14 swersheds to quantify whether or not the wastewater data was significantly leading or lagging the test percent positivity data. The second method involved regressing the percent positivity data on the wastewater data in a negative binomial regression for up to 3 weeks of leading and lagging directions using a one-sided 7-day moving average time-series during each of the increasing, peaking, and decreasing phases. Rate ratios of the increase in test percent positivity per an inter-quartile-range increase in the wastewater indicator were computed for each sewershed. For each lead/lag week, DOHMH then computed a combined estimate across the sewersheds using random-effects metaanalysis.

As shown in Figure 3.2, the test percent positivity data exhibited a strong day-of-week pattern, related to the higher percent positivity on the weekends. This pattern is related to the fact that smaller clinics and doctor's offices are closed on weekends and therefore people who are tested on weekends tend to be sicker individuals who are seen in hospitals, who are more likely to be positive. As shown in Figure 3.3 below, inflection dates for the wastewater data and the percent positivity data were close in time. During the peak phase of the second wave, the percent positivity data led the wastewater data by about two weeks, as shown in the t-test result summarized in the lower right corner of Figure 3.3. For the second peak, a similar pattern was identified. To be used as a leading indicator, wastewater data would need to peak before the percent positivity.

Finally, as shown in Figure 3.4, there is a suggestive pattern indicating the wastewater data led the percent positivity data in the regression analysis during the increasing phase of wave two, but the pattern was imprecise and not consistent across sewersheds, as reflected in their wide confidence intervals. In the peaking and decreasing phases, the wastewater data lags the percent positivity data by about two weeks which is consistent with the result from the first method. Overall, results on this early

dataset do not support the utility of SARS-CoV-2 concentrations in wastewater as a leading indicator of disease surveillance.

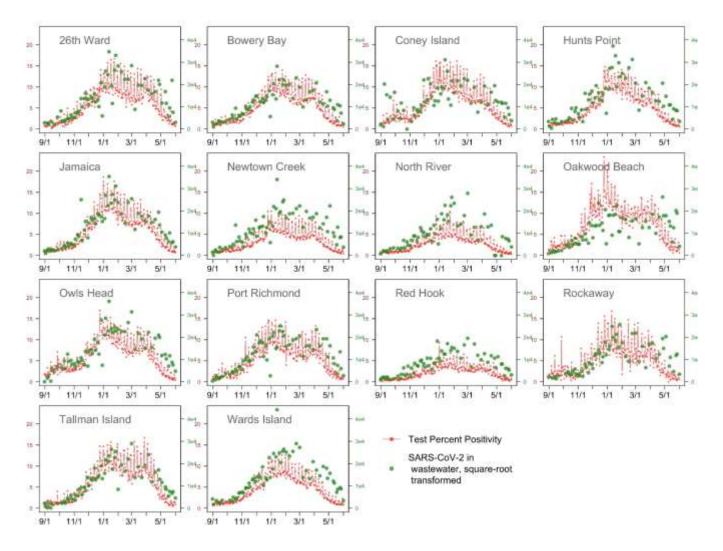


Figure 3.2. Covid-19 PCR test percent positivity (orange) and population-normalized SARS-CoV-2 mRNA copies in wastewater (square-root transformed) in 14 sewersheds from September 2020 through May 2021.

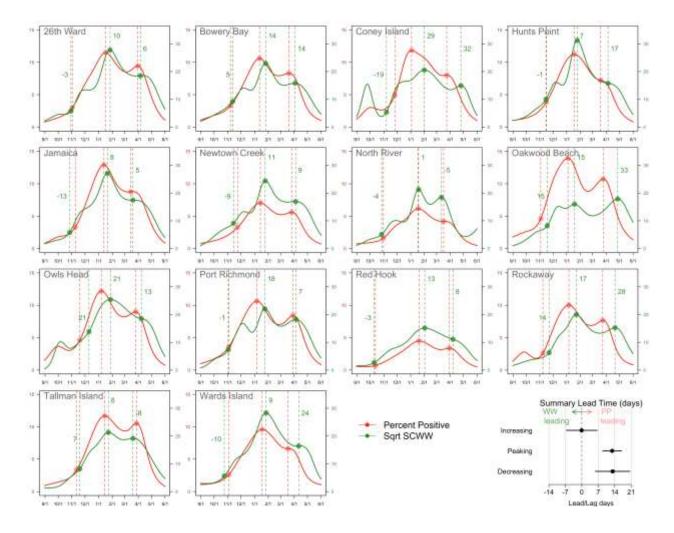


Figure 3.3. Smoothed Covid-19 PCR test percent positivity (in red; left y-axis) and population-normalized SARS-CoV-2 RNA copies in wastewater (in green; right y-axis, square-root transformed and divided by 1000) in 14 sewersheds September 2020 to January 2022. Smoothing was done with natural cubic splines with 10 degrees of freedom over the study period. Dots indicate the dates for inflection (increasing phase); peak (peaking phase); and second peak or shoulder (declining phase). Numbers denote corresponding lead/lag days (negative when the wastewater indicator is leading). A summary of paired t-test across 14 sewersheds shown in the lower right.

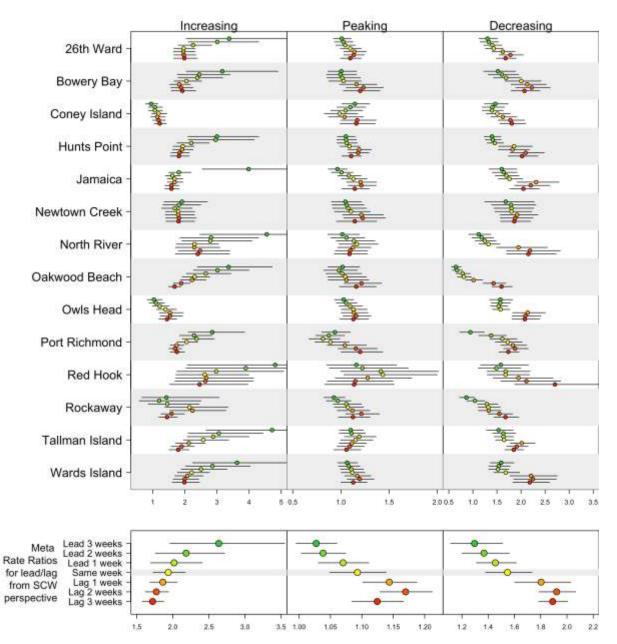


Figure 3.4. Rate ratios (x-axis) of Covid-19 test percent positivity per inter-quartile-increase (in each of the three phase time window) in population-normalized SARS-CoV-2 RNA copies in wastewater (square-root transformed) from 14 sewersheds in the increasing (2020-08-31 to 2020-11-30), peaking (2020-12-01 to 2021-02-28), and decreasing (2021-03-01 to 2021-05-31) periods: (a) top three columns: individual sewersheds results; (b) bottom three columns: random effects combined estimates. The green-to-red color coding denotes lead/lag relationship from SARS-CoV-2 mRNA copies in wastewater (SCWW) perspective (i.e., green: SCWW is leading) as labeled in the plot for combined estimate result.

It should be noted that evaluation of how trends in SARS-CoV-2 RNA levels can be used as a leading indicator is still ongoing. Since the DOHMH's series of quantitative analyses presented in Figures 3.2 - 3.4 was conducted, DEP conducted an extensive study to understand the sources of the strong measurement variability discussed above. This work was carried out with academic partners. The additional study has led to the introduction of significant improvements in the data analysis portion of DEP's process. DEP and DOHMH will continue to examine the utility of this tool. Appendix 6 provides a snapshot of very recent trends that will be the basis of further analysis.

3.2. Cost of Pilot Program

DEP built the WBE program from the ground up at its Newtown Creek laboratory. Operating costs for the program totaled over \$520K for the 21-month period, which translates to almost \$300K annually (Table 3.1). These costs do not reflect program development or the procurement of equipment, which were associated with deployment of capabilities. Those capabilities now exist and are ready to reactivate should the need arise.

Summary of COVID Monitoring Costs - April 2020 through December 2021				
		21 months	Annualized	
DEP Direct Costs	OTPS (100% Consumables)	\$136,264.10	\$77,865.20	
	PS (Laboratory Analysis and Program	\$385,068.95	\$220,039.40	
	total	\$521,333.05	\$297,904.60	

3.3. Analysis of the effectiveness of the pilot program in testing for SARS-CoV-2

The pilot has been highly effective in developing NYC's capacity for WBE, representing an entirely new activity for the DEP Bureau of Wastewater Treatment (BWT) Laboratories. Through collaborations and cross-sectoral groundwork, DEP built a cutting-edge system. This system is capable of measuring SARS-CoV-2 RNA levels and detecting SARS-CoV-2 viral variants in wastewater. DEP and DOHMH are confident in the quality of the SARS-CoV-2 data, which overall correlated with clinical testing data from NYC (Figures 3.1 and 3.2).

Costs for the program reflected the level of investment required to ramp up quickly in response to an unprecedented public health emergency. This expenditure was effective in establishing WBE readiness as part of DEP infrastructure, but entailed significant demands on DEP human resources. DEP will reduce program costs and resource demands by transitioning to testing as part of the CDC National

Wastewater Surveillance System (NWSS) program.^{viii} This program will use a third-party laboratory, LuminUltra, to perform testing at all 14 WRRFs. The program will run for one year and will be paid for by CDC.

The pilot demonstrated the power of collaborations in advancing NYC goals. DOHMH staff provided extensive consultation on interpretation of results and was the primary user consumer of wastewater testing data. DEP and DOHMH jointly participated in the NYC Corona Virus Genomics Collaboration, a group organized in early 2021 by the New York City Health and Hospitals Corporation (H+H), working with the Office of the Mayor. This group included institutions from across NYC that were working on COVD-19 research and testing. The collaboration meetings served as a platform for information exchange, and public health policy updates from the NYC Administration.

DEP also carried out collaborative work with academic researchers in NYC. Professors at New York University Tandon School of Engineering advised onsite, supporting methods development in the laboratory and training staff in analytical procedures. Faculty at City University of New York (CUNY) Queens College, CUNY Queensborough Community College, and the New School for Social Research developed and refined the testing method and led the work on sequencing.

In May 2021, representatives from DEP, DOHMH and researchers from CUNY, NYU and the New School began meeting as the New York City Consortium for the use of Wastewater Surveillance (NYCWS). The NYCWS formalized research relationships and set guidelines for data sharing and involvement of external partners. The stated goal of the NYCWS, which met biweekly, was to enhance the understanding of, and ability to use, wastewater-based epidemiology as a tool in public health in order to protect and promote the health of New Yorkers.

For program development, DEP also consulted with other US wastewater utilities through the Water Research Foundation, in particular drawing on expertise of the Hampton Roads Sanitary District in Virginia Beach, VA. Nearer to home, DEP began providing testing services to wastewater utilities in Westchester County and the City of Plattsburgh in late August 2020.

At the federal level, DEP and DOHMH have been in close contact with the US Department of Health and Human Services (HHS) and CDC, which sponsor NWSS. The data reported by NWSS helps public health officials to better understand the extent of SARS-CoV-2 transmission across the country.^{ix}

In June 2021, DEP partnered with the HHS and CDC to use the services of a third-party contract laboratory (Biobot) as part of a program to test samples from wastewater treatment facilities nationwide. DEP submitted over 240 samples, from June to August 2021. SARS-CoV-2 RNA results were made available to local and state government health agencies through the NWSS portal. Note, DEP's

results were identified by state only; locations of WRRFs were anonymized. In addition, sequencing data were posted onto the National Center for Biotechnological Information website for access to scientists for research purposes. Results from Biobot correlated with results produced by DEP.

3.4. Recommendations to expand the pilot program to include sampling at manhole sites and pumping stations if wastewater-based epidemiology detects SARS-CoV-2 in an amount, as determined by the commissioner of health and mental hygiene, that indicates a localized concentration of COVID-19

Several SARS-Cov-2 mutations not detected in sequencing of clinical specimens in NYC were detected in the sewersheds of Oakwood Beach and Owls Head. DEP and researchers at CUNY initiated a campaign to localize the area where these mutations were detected.^x Personnel from DEP's Pollution Prevention and Monitoring Section designed a sampling approach (Figure 3.6). In this example, the mutation not detected in sequencing of clinical specimens was detected in only one of the nine subsewersheds of Oakwood Beach sampled.

This effort showed that subsewershed monitoring could be used to monitor localized SARS-COV-2 signals. However, this process was very time consuming, because each round of sampling underwent testing to verify results; and planning sampling operations at manholes and pumping stations required reconnaissance in advance and coordination of traffic control. This method would best be used in very limited fashion, over small areas, when a localized measurement is needed. At this time DEP and DOHMH do not recommend pursuing a localized sampling approach.

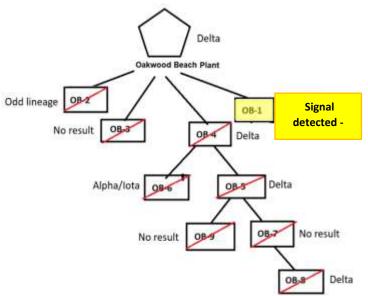


Figure 3.6: Mutation detected in subsampling in the Oakwood Beach sewershed. "Signal detected" refers to instance of a mutation not found in clinical samples.

3.5. Recommendations to extend the pilot program for up to an additional six months if more testing is necessary, as determined by the commissioner, in consultation with the commissioner of health and mental hygiene

Through its partners at CUNY, DEP utilized a targeted sequencing approach (Appendix 4) to identify variants within sewersheds of the WRRFs. This approach only sequenced a portion of the SARS-COV-2 genome, and as such could not distinguish between all the known SARS-COV-2 variants. However, it was able to detect some of the most clinically abundant variants, such as Alpha, Delta, and Omicron in NYC's wastewater. The distributions and trends in variants from wastewater sequences were consistent with NYC clinical SARS-COV-2 sequences (Figure 3.7).

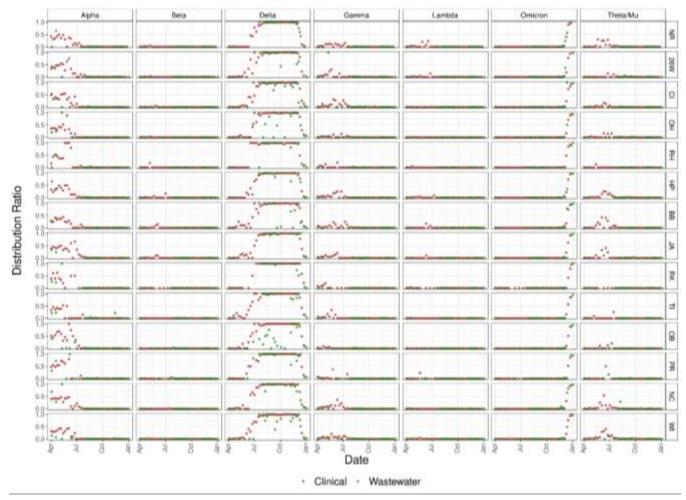


Figure 3.7. SARS-CoV-2 variant distributions in NYC wastewater and clinical sequences from April 2021 to January 2022. Wastewater treatment plant abbreviations: 26W = 26th Ward, CI = Coney Island, OH = Owls Head, RH = Red Hook, BB = Bowery Bay, JA = Jamaica, RK = Rockaway, TI = Tallman Island, PR = Port Richmond, OB = Oakwood Beach, NC = Newtown Creek, WI = Wards Island, NR = North River, HP = Hunts Point

Conclusions

DEP's pilot program to test wastewater from NYC's WRRFs for SARS-CoV-2 established NYC's capacity to monitor disease transmission through its sewersheds. DEP mobilized cutting edge technology and methods to monitor SARS-CoV-2 in 14 sewersheds covering all of New York City. It leveraged partnerships with experts in academia and government to ensure quality and efficiency. As knowledge in this emerging field of public health continues to be advanced, methods will continue to be improved, and additional applications of data from this program may be identified. DEP is committed to maintaining its engagement in this sphere through targeted collaborations with other water utilities, DOHMH, Federal entities and academic partners so WBE can be leveraged further in future public health emergencies.

List of Appendices

Appendix 1: Results of sampling, disaggregated by the site where the sample was collected, date sample was collected, and date sample was tested. Results have been posted to NYC Open Data as "SARS-CoV-2 concentrations measured in NYC Wastewater"

(https://data.cityofnewyork.us/Health/SARS-CoV-2-concentrations-measured-in-NYC-Wastewat/f7dc-2q9f) and the dataset is included with this transmission as an excel file.

Appendix 2: Hoar, C., Chauvin, F., Katehis, D., Clare, A., McGibbon, H., Castro, E., Patinella, S., Dennehy, J.J., Trujillo, M., Smyth, D., Silverman, A.I. *In revision*. "Monitoring SARS-CoV-2 in wastewater during New York City's second wave of COVID-19: Sewershed-level trends and relationships to publicly-available clinical testing data." A pre-print is available on medrxiv (accessible at https://www.medrxiv.org)

Appendix 3: Smyth, D.S., Trujillo, M., Cheung, K., Gao, A., Hoxie, I., Kannoly, S., Kubota, N., Markman, M., San, K., Sompanya, G. and Dennehy, J.J., 2021. "Detection of Mutations Associated with Variants of Concern Via High Throughput Sequencing of SARS-CoV-2 Isolated from NYC Wastewater." *medRxiv*; and Smyth, D.S., Trujillo, M., Gregory, D.A., Cheung, K., Gao, A., Graham, M., Guan, Y., Guldenpfennig, C., Hoxie, I., Kannoly, S. and Kubota, N., 2021. "Tracking Cryptic SARS-CoV-2 Lineages Detected in NYC Wastewater." *medRxiv*

Appendix 4: Gregory, D.A., Wieberg, C.G., Wenzel, J., Lin, C.H. and Johnson, M.C., 2021. "Monitoring SARS-CoV-2 populations in wastewater by amplicon sequencing and using the Novel Program SAM Refiner." *Viruses*, 13(8), p.1647.

Appendix 5: Methods

Appendix 6: Summary of SARS-CoV-2 wastewater data for New York City's 14 sewersheds for the Omicron wave (November 2021 to January 2022)

End notes

ⁱ Albert, S., Ruíz, A., Pemán, J., Salavert, M. and Domingo-Calap, P., 2021. Lack of evidence for infectious SARS-CoV-2 in feces and sewage. *medRxiv*.

ⁱⁱ Aguiar-Oliveira, M.D.L., Campos, A., R Matos, A., Rigotto, C., Sotero-Martins, A., Teixeira, P.F. and Siqueira, M.M., 2020. Wastewater-Based Epidemiology (WBE) and Viral Detection in Polluted Surface Water: A Valuable Tool for COVID-19 Surveillance—A Brief Review. *International journal of environmental research and public health*, *17*(24), p.9251.

^{III} . New York City Department of Health and Mental Hygiene. 2021. "COVID-19: Data," <u>https://www1.nyc.gov/site/doh/covid/covid-19-data-totals.page</u>, accessed 1/24/2022

^{iv} . Text taken directly from Local Law 28 of 2021; reflects terminology and capitalization in the Law.

^v. Text taken directly from Local Law 28 of 2021; reflects terminology and capitalization in the Law.

^{vi} . Text taken directly from Local Law 28 of 2021; reflects terminology and capitalization in the Law.

^{vii} . Sampling and analysis has continued into 2022. For updates, see: <u>https://www1.nyc.gov/site/doh/covid/covid-19-data-totals.page</u>

viii . Centers for Disease Control and Prevention. 2022. "National Wastewater Surveillance System (NWSS): A new public health tool to understand COVID-19's spread in a community" <u>https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html</u> Accessed February 10, 2022

^{ix} Centers for Disease Control and Prevention. 2021. "COVID Data Tracker," <u>https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html</u>, accessed 12/21/2021.

^x Smyth, D.S., Trujillo, M., Gregory, D.A., Cheung, K., Gao, A., Graham, M., Guan, Y., Guldenpfennig, C., Hoxie, I., Kannoly, S. and Kubota, N., 2021. "Tracking Cryptic SARS-CoV-2 Lineages Detected in NYC Wastewater." *medRxiv*

Appendix 1

Results of sampling, disaggregated by the site where the sample was collected, date sample was collected, and date sample was tested

Results have been posted to NYC Open Data as "SARS-CoV-2 concentrations measured in NYC Wastewater" (<u>https://data.cityofnewyork.us/Health/SARS-CoV-2-concentrations-measured-in-NYC-Wastewat/f7dc-2q9f</u>) and the dataset is included with this transmission as an excel file, entitled "Appendix_1_COVID19_SARS-CoV-2_data_on_wastewater_samples__DATASET_V01.00".

Appendix 2

1 Title

2 Monitoring SARS-CoV-2 in wastewater during New York City's second wave of COVID-3 **19:** Sewershed-level trends and relationships to publicly available clinical testing data

4

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26 Abstract

27

New York City's ongoing wastewater monitoring program tracked trends in sewershed-level 28

- 29 SARS-CoV-2 loads starting in the fall of 2020, just before the start of the City's second wave of
- 30 the COVID-19 outbreak. During a five-month study period, from November 8, 2020 to April 11,
- 2021, viral loads in influent wastewater from each of New York City's 14 wastewater treatment 31
- plants were measured and compared to new laboratory-confirmed COVID-19 cases for the 32
- 33 populations in each corresponding sewershed, estimated from publicly available clinical testing
- 34 data. We found significant positive correlations between viral loads in wastewater and new
- COVID-19 cases. The strength of the correlations varied depending on the sewershed, with 35
- Spearman's rank correlation coefficients ranging between 0.38 and 0.81 (mean = 0.55). Based on 36
- a linear regression analysis of a combined data set for New York City, we found that a 1 log₁₀ 37
- 38 change in the SARS-CoV-2 viral load in wastewater corresponded to a $0.6 \log_{10}$ change in the
- 39 number of new laboratory-confirmed COVID-19 cases/day in a sewershed. An estimated minimum detectable case rate between 2 - 8 cases/day/100,000 people was associated with the 40
- method limit of detection in wastewater. This work offers a preliminary assessment of the 41
- 42 relationship between wastewater monitoring data and clinical testing data in New York City.
- 43 While routine monitoring and method optimization continue, information on the development of
- 44 New York City's ongoing wastewater monitoring program may provide insights for similar
- 45 wastewater-based epidemiology efforts in the future.
- 46

47 Introduction

48

49 In March 2020, New York City became an epicenter of the coronavirus disease 2019 (COVID-

- 50 19) pandemic. In response to this first wave of COVID-19 cases, the New York City Department
- of Environmental Protection (NYC DEP) the city agency responsible for wastewater collection
- 52 and treatment launched a wastewater monitoring program with the goal of tracking sewershed-
- 53 level trends in the concentration of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-
- 54 CoV-2), the virus that causes COVID-19. The program was developed in partnership with
- 55 researchers at New York University, Queens College, Queensborough Community College, and
- 56 The New School, with all routine analysis conducted in the NYC DEP's existing microbiology
- 57 laboratory under the management of the NYC DEP.
- 58
- 59 Wastewater-based epidemiology (WBE) programs for COVID-19, including the one in New
- 60 York City (NYC), were established on the premise that SARS-CoV-2 virions are excreted in the
- 61 human waste of individuals infected with SARS-CoV-2 and that the resulting concentrations of
- 62 viral RNA measured in wastewater are indicative of disease incidence or prevalence in the
- 63 contributing sewershed. Significant associations between SARS-CoV-2 RNA concentrations
- 64 measured in wastewater and metrics of COVID-19 disease incidence--including case rates--have
- been shown at scales ranging from single buildings to entire sewersheds.^{1–3} Early reports from
- 66 WBE programs suggested promising predictive applications that could help inform COVID-19
- 67 response measures,^{4,5} sparking widespread interest in SARS-CoV-2 monitoring programs around
- 68 the world.^{6,7} While the extent to which wastewater data is a leading indicator of trends in
- 69 COVID-19 incidence ahead of clinical data may vary depending on clinical testing rates,^{8,9} WBE
- data do offer the advantage of providing information representative of entire populations, free
 from clinical testing-related biases. In NYC, where communities of color and high-poverty areas
- were disproportionately impacted by the first wave of the COVID-19 pandemic,¹⁰ testing rates
- 72 varied spatially, with significant demographic-based disparities.¹¹ In situations where clinical
- 74 testing does not adequately sample vulnerable populations. WBE may help inform modifications
- 75 to testing strategies and provide supplemental information regarding COVID-19 trends.
- 76 Wastewater monitoring is therefore a potential tool to identify new outbreaks of COVID-19 after
- high clinical testing rates associated with major "waves" of disease incidence have subsided or
- 78 when resources and technical capacity for extensive clinical testing of individuals are limited.
- 79

80 These opportunities make WBE an attractive option for many municipalities, including NYC, to

- 81 confirm findings from clinical testing about population-level COVID-19 dynamics and to
- 82 monitor for new outbreaks in instances when testing is inadequate. In August 2020, the NYC
- 83 DEP's SARS-CoV-2 wastewater monitoring program began routine analysis of influent
- 84 wastewater collected from NYC's 14 wastewater treatment plants (referred to as wastewater
- 85 resource recovery facilities (WRRF) by the NYC DEP) (SI Table S1), capturing data during the
- region's second wave of COVID-19 cases, which started in the fall of 2020. The sewershed
 catchment areas contributing to each of the 14 WRRFs vary markedly in size, serving
- populations ranging from approximately 120,000 to 1.2 million residents. To assess the
- relationship between NYC sewershed-level SARS-CoV-2 RNA concentrations and confirmed
- relationship between NTC sewershed-rever SAKS-Cov-2 KNA concentrations and commend
 cases of COVID-19 within each sewershed, wastewater data were compared to publicly available
- 91 case data provided by the NYC Department of Health and Mental Hygiene (DOHMH). In
- 92 presenting findings from the NYC DEP, we also aim to provide insights into the development of

a sustainable wastewater monitoring program designed for long-term, routine tracking of trendsin virus loads for multiple sewersheds serving a large urban population.

95

96 Methods

97

98 Sample collection and processing

99 24-h flow-weighted composite influent wastewater samples were collected from each of NYC's 100 14 WRRFs twice weekly beginning August 31, 2020. From January 31, 2021 to April 18, 2021 sampling was reduced to once weekly. Each composite sample consisted of eight grab samples 101 102 collected every three hours beginning at 7:00 AM on the sampling date. Samples were 103 transported on ice and stored at 4 °C until processing, which started within twelve hours after the final grab sample was collected. For each sampling date, one of the 14 samples was analyzed in 104 duplicate and the remainder were analyzed as single samples; facilities were selected for 105 106 duplicate analysis on a rotating basis. A method blank containing Type I deionized water was 107 included with each set of samples to confirm the absence of contamination during sample processing. Detailed descriptions of materials, methods, and data analysis are provided in the SI. 108 109 In brief, 40-mL aliquots of the 24-h composite samples were first pasteurized (60 °C, 90 min), and then centrifuged (5000 x g, 4 °C, 10 min) to remove solids. The supernatant was filtered 110 (0.22 µm, cellulose acetate) and then subjected to virus concentration using polyethylene glycol 111 112 (PEG) precipitation (addition of 4.0 g PEG and 0.9 g NaCl followed by overnight incubation at 4

^oC, and centrifugation at 12,000 x g at 4 °C for 120 min to pellet viruses).¹² The supernatant was

discarded and RNA was extracted from the concentrated PEG pellet using the Qiagen QiaAmpViral RNA Mini Kit with modifications (described in the SI).

116

117 SARS-CoV-2 quantification by RT-qPCR

118 A one-step RT-qPCR assay was used to quantify copies of the SARS-CoV-2 nucleocapsid (N)

gene, targeting the N1 region (CDC RUO Primers and Probes, Integrated DNA Technologies¹³)

120 in triplicate reactions on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific).

121 Synthetic SARS-CoV-2 RNA covering > 99.9% of the viral genome (Twist Bioscience Control

122 1, GENBANK ID MT007544.1) served as both a positive control and standard used in a decimal

- serial dilution for quantification of N1 gene copies.
- 124

The limit of detection (LOD) and limit of quantification (LOQ) for the assay were estimated 125 from replicate standard curves as described by Forootan et al. 2017¹⁴ and found to be 4,500 126 127 copies/L and 15,000 copies/L, respectively. Note that these LOD and LOQ values as well as calculated sample concentrations are relative to the approximate concentration of the synthetic 128 RNA control reported by the manufacturer, as absolute quantification of the RNA control was 129 130 not feasible when sample analysis began. Note that quantification of the RNA control through 131 digital PCR is underway. N1 concentrations--including those of the LOD and LOQ--reported in 132 the current version of this work may therefore be updated in future versions to reflect the 133 quantified concentration of the RT-qPCR standard. Nonetheless, while the approach described 134 herein limits direct comparison of N1 concentrations to those found in other studies, it does not 135 alter trends and comparisons across facilities examined within this study. In addition, we elected to use a pooled standard curve to quantify samples on all plates to ameliorate variability in 136 137 standard preparation by different analysts from plate to plate. A description of the analysis used 138 to motivate this decision is presented in the SI (Figure S1). The absence of contamination during

- 139 RT-qPCR preparation was confirmed through no template controls included on all RT-qPCR
- 140 plates. Only samples quantified above the LOQ were included in subsequent analysis. From
- 141 September 8, 2020 to June 8, 2021, samples were collected from each facility on 72 sampling
- dates, with samples from only two dates associated with method blanks having N1
- 143 concentrations above the LOD; samples collected on these two dates were flagged as
- 144 contaminated and were not included in subsequent analysis.
- 145
- 146 An attenuated bovine coronavirus (BCoV) (Calf-Guard® Bovine Rota-Coronavirus Vaccine,
- 147 Zoetis) was used as a process control.^{15,16} BCoV was inoculated into samples after the
- pasteurization step (details provided in the SI). A one-step RT-qPCR assay, adapted from
 previously published assays,^{15–17} targeting the transmembrane-protein gene of BCoV was used to
- previously published assays,^{15–17} targeting the transmembrane-protein gene of BCoV was used to qualitatively assess BCoV recovery for each sample using an aliquot of the extracted RNA
- 151 (primers and probes purchased from Integrated DNA Technologies). Detection of BCoV was
- used to confirm whether viruses were recovered in samples for which the N1 target was not
- detected. Additional details regarding the RT-qPCR assays, standard curves, and QA/QC
- 154 procedures are provided in the SI.
- 155
- 156 Data analysis

157 The concentration of the N1 RNA target in wastewater (C_{WW}) was determined for each sample in 158 units of N1 gene copies (GC)/L according to Equation 1, where N_r is the number of N1 GC 159 measured by RT-qPCR, $V_{RNA,s}$ is the volume of RNA extracted from each sample (60 µL), 160 $V_{RNA,r}$ is the volume of template RNA added to the RT-qPCR reaction (5 µL), and V_s is the 161 volume of wastewater sample analyzed (0.04 L).

162

163
$$C_{WW} = (N_r \times V_{RNA,s})/(V_{RNA,r} \times V_s)$$

164

The resulting C_{WW} was then normalized by the associated daily influent wastewater flow rate 165 (i.e., the flow rate in the same facility on the same day) to calculate the SARS-CoV-2 viral 166 loading rate (L_{WW}) in units of N1 GC/day (Equation 2). Given that 60% of the NYC sewer 167 system is a combined stormwater-sewer system, flow-based normalization was used to account 168 for differences in per capita water usage and variability in wastewater flow rates caused by non-169 domestic water inputs (e.g., rain events), which can affect measured virus concentrations. In 170 171 Equation 2, Q is the daily flow rate at the facility in millions of gallons per day (MGD), and CF is the conversion factor required to convert from liters to million gallons $(3.78541 \times 10^{6} \text{L/MG})$. 172 173 Continuous measurements of flow rate were conducted at each facility using either magnetic flow meters or flow measuring weirs (with uncertainty in measurements of \sim 5%). Average daily 174 175 flow rates had been measured at each facility prior to the establishment of the SARS-CoV-2 176 monitoring program, and thus required no additional analysis burden, making it a logistically 177 advantageous option for normalization of virus measurements.

178

$$179 \qquad L_{WW} = C_{WW} \times Q \times CF$$

180

181 Statistical analyses of relationships between SARS-CoV-2 loads in wastewater and laboratory-

- 182 *confirmed COVID-19 cases*
- 183 Relationships between SARS-CoV-2 wastewater data in each sewershed and laboratory-
- 184 confirmed COVID-19 cases for the associated sewershed population were evaluated through

Equation 1

Equation 2

correlation and linear regression analyses. Clinical data were obtained from publicly available 185 data provided by the NYC DOHMH.¹⁸ In particular, the data set "last7days-by-modzcta.csv", 186 which was posted online daily, was used to obtain daily reports of the cumulative clinical 187 188 molecular testing results over the previous seven days for each modified ZIP code tabulation area (MODZCTA) in NYC.¹⁸ Specifically, data on the total clinical COVID-19 tests administered and 189 the total number of positive tests (not including individuals who previously tested positive), 190 191 reported based on date of specimen collection, were obtained. Note that molecular tests included 192 diagnostic PCR tests and did not include antigen or antibody tests. This data set was used to 193 calculate 7-day averages of new COVID-19 cases (i.e., positive molecular tests) per day, 194 organized by the last date in the 7-day range. For example, the 7-day average reported on 195 February 14 represents the daily average of new cases calculated based on the total number of positive molecular tests collected from February 8 to February 14. Data were available starting 196 197 on November 7, 2020, with data from March 15, 2021 to March 21, 2021 omitted due to 198 technical issues related to data transmission during this period (Figure S.2). While alternative 199 data sets were available with cumulative new COVID-19 case counts prior to November 2020, 200 these data were organized by the date that test results were reported, as opposed to date of 201 specimen collection, and were therefore not recommended by NYC DOHMH for use in 202 calculating the number of daily new COVID-19 cases.¹⁸

203

Each of the 177 MODZCTAs were assigned to one of NYC's 14 sewersheds. Of the 177

MODZCTAs, 44 straddled multiple sewershed areas and were assigned to only the sewershed in which it had the greatest overlapping land area. Total new cases in each sewershed each day were calculated by summing new cases in the MODZCTA assigned to that sewershed. The same data set was used to calculate 7-day averages of COVID-19 testing rates (i.e., the number of tests administered divided by the total population) and the percentages of COVID-19 tests that were positive for each sewershed (Figure S.2).

Spearman correlations between SARS-CoV-2 viral loading rates in wastewater (N1 GC/day) and 7-day averages of new daily COVID-19 cases were determined for each individual sewershed for a five-month study period (November 8, 2020 to April 11, 2021). Correlations were also determined for a combined data set that included each data pair (i.e., SARS-CoV-2 viral loading rates and 7-day average of new COVID-19 cases on each date) for all facilities, excluding the Dert Bishmond and Onlywood Boach WBPErs (see the Bogulta and Disputsion section). For the

Port Richmond and Oakwood Beach WRRFs (see the Results and Discussion section). For the
 combined data, correlations were also evaluated after removing data pairs associated with

218 combined data, correlations were also evaluated after removing data pairs associated with 219 potentially inadequate clinical testing rates: data for dates with percentages of positive molecular

219 potentially inadequate clinical testing rates: data for dates with percentages of positive molecular 220 tests (7-day average) that exceeded 10% in the sewershed were excluded. A general benchmark

suggested by the World Health Organization in the Spring of 2020 indicated that clinical testing

is less likely to represent all infections in a population when the percentage of positive tests

exceeds approximately 10%;^{19,20} we therefore excluded these data in an effort to best

- approximate the incidence of SARS-CoV-2 infections.
- 225

226 To assess whether trends in SARS-CoV-2 viral loading rates in wastewater preceded trends in

clinical testing data, correlations between the two data sets were also evaluated for each

sewershed with the clinical data shifted back in time with lags ranging from 0 to 21 days. For

this analysis, additional clinical data from April 12, 2021 to May 2, 2021 was included to

230 maintain a constant number of data pairs for each number of lag days applied.

С	С	1
Ζ	Э	т

232 Simple linear regressions were performed using log₁₀-transformed SARS-CoV-2 viral loading rates (N1 GC/day) and log₁₀-transformed 7-day averages of new COVID-19 cases (new COVID-233 234 19 cases/day) for each individual sewershed as well as for the combined data set. The combined data set was assessed with and without the testing rate filter described above. Linear regressions 235 236 were used to estimate the equivalent number of cases/day/100,000 people associated with the method LOD (C_{LOD}), equal to 4,500 N1 GC/L. This estimate was calculated for each facility 237 using individual, sewershed-specific linear regressions and using the linear regression for the 238 combined data set. First, the LOD was converted to a SARS-CoV-2 viral loading rate in 239 wastewater $(L_{WW,LOD})$ for each sewershed in units of N1 GC/day using Equation 3, where Q_{ava} 240 is the average of daily flow rates at the facility over the study period (Table S.1), in MGD. 241 242 243 $L_{WW,LOD} = C_{LOD} \times Q_{ava} \times CF$ Equation 3

244

245 $L_{WW,LOD}$ for each sewershed were then input to the linear regressions determined for each 246 sewershed to estimate the number of new COVID-19 cases/day associated with the SARS-CoV-247 2 method LOD (*Case_{LOD}*), using Equation 4, where *m* and *b* are the slope and y-intercept of the 248 linear regression line, respectively (presented for each sewershed in the Results and Discussion 249 section). An example estimation is illustrated graphically in Figure S.6. Resulting *Case_{LOD}* values 250 were normalized per 100,000 people using MODZCTA-level population estimates from the 251 NYC DOHMH NYC Coronavirus Disease 2019 (COVID-19) Data.¹⁸

252

253 254 $log_{10}(Case_{LOD}) = m \times log_{10}(L_{WW,LOD}) + b$

As described above, quantification of the RT-qPCR standard for the N1 target is underway. Future updates to the N1 standard concentration will change the reported method LOD, in units of N1 GC/L. However, because all sample concentrations will also be adjusted to reflect the updated standard concentration, we anticipate that the resulting relationships between the wastewater data and the clinical data (including the associated $Case_{LOD}$) should remain similar to what is reported herein.

261

Statistical analyses were performed using R, and figures were created using GraphPad Prism.^{21,22}
 263

265

265 Results and Discussion

266

267 Methodological considerations for SARS-CoV-2 quantification in wastewater

268 The public health emergency caused by the emergence of COVID-19 required the expedited

269 development of NYC DEP's SARS-CoV-2 wastewater monitoring program. As such, several

270 methodological choices for virus quantification were considered, and the ultimate standard

operating procedure (SOP) described herein was developed reflecting NYC DEP's program

272 goals of monitoring trends in SARS-CoV-2 viral loads in wastewater, accounting for equipment

availability, existing expertise of personnel, and considerations of material procurement.

274 Selections were also made to minimize analyst-based variability. For example, commercially-

available kits for RNA extraction were considered over alternatives that may be more sensitive to

analyst skill and consistency. Data analysis and internally-developed QA/QC guidelines were

Equation 4

established in line with programmatic goals. Additional methodological considerations, such asthe inclusion of a filtration step in sample preparation, are discussed in the SI.

279

Long-term routine monitoring to assess virus trends through quantification with RT-qPCR
 requires reliable comparison of data originating from different RT-qPCR plates prepared by

different analysts, which presents several challenges. First, in the absence of a formally

283 quantified standard for the N1 RNA target, this program relied on the use of a synthetic RNA

control. An approximate concentration of this RNA control was provided by the manufacturer,

but was found to differ between lots purchased at different times. In addition, standard curves for

routine RT-qPCR assays were prepared by different analysts on different days, with separate
 serial dilutions of standards performed for each individual RT-qPCR plate. To account for any

resulting variability caused by these aspects of the RT-qPCR quantification method, we

quantified the concentration of each RNA control lot relative to the original lot used and applied

a pooled standard curve for quantification of all samples (Figure S.1). Challenges associated with

291 RT-qPCR-based quantification using a standard curve highlight the benefits of alternative

methods, such as digital PCR for absolute RNA quantification, which eliminates the need for a

standard curve and may offer more sensitive detection for environmental samples.²³ Nonetheless,

the methodology employed in this work allowed us to compare relative viral loads and

- confidently assess of trends of SARS-CoV-2 in wastewater over time.
- 296 297

298 SARS-CoV-2 viral loads in influent wastewater

SARS-CoV-2 viral loads in NYC's 14 sewersheds between September 8, 2020 and June 8, 2021 were determined from quantifiable N1 gene copy (GC) concentrations in influent samples and are presented normalized by sewershed population (Table S.1²⁴) in Figure 1. Maximum population-normalized SARS-CoV-2 viral loads for each facility during this period ranged from 1.6×10^8 to 6.8×10^8 N1 GC/day/population, with many of these values occurring around the

time when a peak in COVID-19 cases was observed (January 2021). Note that in September of

305 2020, prior to the increase in COVID-19 cases associated with NYC's second wave of the

306 outbreak, N1 concentrations in wastewater remained below the LOQ in several sewersheds.

307

308 Visual inspection of trends in SARS-CoV-2 quantities in wastewater and new laboratory-

309 confirmed COVID-19 cases indicates an association between the wastewater and clinical data.

310 The strength of this association varied across sewersheds, as reflected in results from statistical

analysis presented in the next section. Additionally, most sewersheds exhibited peaks for both

data sets in January 2021 (Figure 1), with two notable exceptions being Oakwood Beach and

Port Richmond, discussed below. Sewersheds with lower incidence rates of COVID-19 (e.g.,

Red Hook WRRF) generally had lower per capita SARS-CoV-2 viral loads in wastewater than

those with higher incidence rates of COVID-19 (e.g., Hunts Point WRRF).

316

317 SARS-CoV-2 viral loads in the Coney Island WRRF influent in September 2020 and October

318 2020 displayed a high degree of variability, with some measured virus loads that were greater

than those in all other sewersheds during that period, despite a consistent processing method

applied for all samples and confirmed COVID-19 case rates that were consistently low across

321 NYC (Figure 1). While there were relatively low rates of clinical testing in New York City in

322 September 2020 and COVID-19 clusters emerged in some neighborhoods served by the Coney

Island WRRF at that time,²⁵ it is unclear if these factors contributed to the high viral loads
measured in some Coney Island WRRF samples. For example, COVID-19 clusters were also
identified in other sewersheds at this time, yet did not result in high SARS-CoV-2 loads in
influent samples collected from other WRRFs, and it is difficult to determine whether clinical
testing was adequate. It should also be noted that given its large geographic resolution,
sewershed-level monitoring may not fully capture the effect of disease clusters (such as those

identified at high spatiotemporal resolution using clinical data²⁶) that may be relatively small
 compared to the sewershed or may straddle multiple sewersheds. Though not examined in this

- 331 work, differences in wastewater quality or sewershed characteristics may also have contributed
- to the observed variability.
- 333

A smaller extent of variability in measured SARS-CoV-2 viral loads was observed to varying

- degrees across all facilities and can stem from several sources. Evaluation of duplicate samples
 analyzed during the study period allowed for an assessment of potential variability due to sample
- 336 analyzed during the study period anowed for an assessment of potential variability due to sample 337 processing and RNA quantification. Relative standard deviations for N1 concentrations of
- duplicate samples (i.e., the standard deviation of concentrations from duplicate samples, each
- 339 with triplicate RT-qPCR reactions, as a percent of the average concentration) ranged from 3% to
- 44% (mean = 17%, median = 14%); these values are comparable to those reported elsewhere for
- measurement of N1 concentrations in influent wastewater.^{16,27} Aside from methodological
- sources of variability, potential sources of variability or uncertainty include (1) dilution of
- wastewater chemical composition, which may interfere with sample processing or RNA
- quantification methods, (3) variability in SARS-CoV-2 shedding intensity and duration for
 infected individuals^{28–30} and (4) the extent and consistency of viral RNA degradation in
 sewers.^{27,31}
- 348

349 To account for variability in wastewater flow rates and minimize the effect of (1), viral loads 350 calculated using measured wastewater flow rates (Equation 2) were used for analysis instead of 351 N1 concentrations. Preliminary tests with an RT-qPCR inhibition control assay during method 352 optimization were used to assess the impact of factor (2) and indicated minimal inhibition (data 353 not shown). Regular assessment of inhibition with additional control assays was not feasible 354 during routine monitoring due to resource constraints. In addition, dilution of RNA, a strategy 355 used to reduce PCR inhibition, was avoided in order to maintain consistency in sample processing, given that viral concentrations in samples collected during periods of low COVID-19 356 357 case rates were susceptible to dilution below the limits of quantification or detection. While not included in this work, assessment of viral recovery and wastewater matrix effects should be 358 considered for future research aiming to characterize uncertainty in WBE data. Although beyond 359 360 the scope of this work, identifying and characterizing external factors related to (3) and (4) is the focus of ongoing SARS-CoV-2 WBE research efforts. Considering these uncertainties and 361 variabilities in wastewater data, which likely increase with scale,³² we did not attempt to quantify 362 the number of SARS-CoV-2 infections in each sewershed based on wastewater data, but instead 363 364 explored the relationship between viral quantities in wastewater and publicly available clinical 365 data to assess trends and associations, and examine differences between sewersheds. 366

As mentioned above, SARS-CoV-2 viral loads in wastewater from the Port Richmond and
Oakwood Beach WRRFs (both located in the borough of Staten Island) did not capture the peak

369 in COVID-19 cases that was observed in January 2021 across all sewersheds. In the Port 370 Richmond and Oakwood Beach sewersheds there was a marked increase in COVID-19 cases in 371 December 2020 that was accompanied by an associated peak in the SARS-CoV-2 viral load in 372 wastewater during this time. However, as new COVID-19 cases in Staten Island increased by 373 60% in January 2021, the virus loads in wastewater stayed constant or decreased. Compared to 374 sewersheds in the other boroughs, those in Staten Island had relatively high clinical test 375 positivity in December and January (7-14%), despite having an average testing rate (i.e., number 376 of clinical tests administered per capita) for the study period that was greater than that of over 377 half of the other sewersheds (Figure S.2). This observation suggests that testing may not have 378 adequately captured all infections in Staten Island during this period. While inadequate clinical 379 testing rates could potentially reduce the accuracy of the observed relationships between clinical and wastewater data for these sewersheds, it does not explain the lower-than-expected SARS-380 CoV-2 viral loads measured in Staten Island wastewater in January 2020. A more likely 381 382 explanation could stem from the composition or operation of the wastewater system in the borough. For example, a portion of the Staten Island population is not served by the sewer 383 384 system and instead uses septic systems. As such, a segment of this population does not contribute 385 to the sewer system, and viruses excreted by these residents would not have been present in the 386 influent wastewater at the Oakwood Beach and Port Richmond WRRFs. Nonetheless, given that 387 the population served by septic systems on Staten Island is thought to be smaller than those 388 served by the sewer system, it is unlikely that this hypothesis can entirely explain the 389 discrepancy between measured SARS-CoV-2 viral load and new COVID-19 cases. In addition, 390 much of Staten Island uses separated rather than combined stormwater-sewer systems, which 391 could potentially impact the wastewater matrix and influence viral recovery during concentration 392 and quantification steps in sample analysis. Because of these discrepancies, the Staten Island 393 sewersheds were excluded from analysis of the combined data set and the estimation of 394 minimum COVID-19 case rates associated with the LOD.

395

By early June 2021, city-wide weekly averages of the percentage of positive COVID-19 clinical tests declined below 1%, and over 50% of NYC residents had received at least one dose of a COVID-19 vaccine.^{18,33} To minimize the potential impact of mass vaccination on the evaluation of relationships between case rates and SARS-CoV-2 concentrations in wastewater presented in this work, we chose to conduct the statistical analyses described in the following section for a period ending in early April, shortly after New York State extended vaccination availability to individuals of 16 years and older.

403

404 *Relationships between SARS-CoV-2 viral loads in wastewater and new laboratory-confirmed* 405 *COVID-19 cases*

- 406 Significant positive correlations between SARS-CoV-2 viral loads in wastewater and new
- 407 laboratory-confirmed COVID-19 cases in the corresponding populations were found for all
- 408 individual sewersheds and for the combined data set (Spearman, p < 0.05), indicating, as
- 409 expected, that an increase in COVID-19 cases was associated with an increase in SARS-CoV-2
- 410 concentrations in wastewater (Figure 2). Correlation coefficients (ρ) for the individual
- 411 sewersheds ranged from 0.38 (Coney Island WRRF) to 0.81 (Wards Island WRRF), with an
- 412 average of 0.55. Similar correlation coefficients between SARS-CoV-2 wastewater
- 413 concentrations and clinical case data have been reported elsewhere.^{16,34} Note that analysis of
- 414 correlations between virus concentrations (N1 GC/L, as opposed to virus loads) and new

415 COVID-19 case rates (cases/day/100,000, as opposed to cases/day) yielded similar results (Table 416 S.3). The correlation coefficient for the combined data set ($\rho = 0.82$) was higher than for any of

- 416 S.3). The correlation coefficient for the combined data set ($\rho = 0.82$) was higher than for a 417 the individual sewersheds (Figure 3.a).
- 418

Minimal differences were observed in the magnitudes of the Spearman's rank correlation 419 coefficients between clinical COVID-19 case data and SARS-CoV-2 viral loads in wastewater 420 for the data sets with and without lag times applied (Figure S.4). Furthermore, correlations for 421 several sewersheds--including the Wards Island WRRF--were strongest without a time lag 422 423 between the two data sets. Previous studies, applying a variety of assessment methods, have 424 suggested lag times between clinical testing and wastewater data ranging on the order of days to 425 weeks, while others have indicated that the SARS-CoV-2 concentration in wastewater is not a leading indicator of COVID-19 diagnosis.⁹ Inconsistent findings for lag times may be attributed 426 to whether clinical data are presented by the date of specimen collection or the date that results 427 428 are reported, as well as the adequacy of COVID-19 testing rates, which vary in different regions 429 and shift across time. Clinical data collected during periods with low testing rates are less likely 430 to capture all infections in a region, and individuals may be more likely to be tested after 431 symptom onset, at a time when viral shedding in feces may have already begun. These conditions can result in a lag behind wastewater monitoring data, which provides viral load 432 information independent from clinical testing rates. Data for this work was collected during a 433 434 time when testing rates were significantly higher than those during the first wave of the pandemic in NYC, and weekly median turnaround times for test results were 1 to 2 days.¹⁸ 435 436 Furthermore, we could not confidently rule out that the small improvements in correlations 437 observed when applying a lag time for some sewersheds was an artifact of variability in the 438 measured wastewater data. A rigorous assessment of lag time would also need to account for 439 contributions of previous as well as newly infected individuals to viral loads in wastewater, 440 which was beyond the scope of this work. For these reasons, we considered data without a time

- 441 lag for subsequent comparisons and linear regression analysis.
- 442

443 Because the nonparametric Spearman's rank correlation was used for this analysis, results 444 suggest that there is, at minimum, a monotonic, direct relationship between SARS-CoV-2 quantified in wastewater and clinically confirmed COVID-19 cases. Linear relationships 445 446 between the two log₁₀-transformed datasets were assessed through analysis of linear regressions, 447 with the best fit found for the Wards Island WRRF ($R^2 = 0.65$) and some of the poorest fits found for the sewersheds in Staten Island (Figure 2). Inconsistent relationships between sewershed-448 level SARS-CoV-2 viral loads in wastewater and COVID-19 cases observed across sewersheds 449 may be due to differences in the sewer systems for each sewershed, including sewershed areas, 450 451 residence times of wastewater in the sewer system, the presence of non-domestic wastewater inputs, proportions of the population made up by transient individuals or commuters, and per 452 453 capita water use. Differences could also be related to clinical testing rates for each sewershed, 454 though no significant correlation was found between the slopes of the linear regression lines and the average testing rates for the study period for each sewershed (Spearman, p > 0.05). Similarly, 455 456 no significant correlations were found between the slopes of the linear regression lines and (1) 457 average wastewater flow rate, (2) sewershed population, or (3) average per capita wastewater flow rate (Spearman, p > 0.05), which was expected given that N1 concentrations were 458 459 normalized by flow rate. Nonetheless, the linear regression found using the combined data set had a strong fit ($R^2 = 0.70$) relative to the fits of regressions for the individual sewersheds. 460

461

462 Understanding the utility of SARS-CoV-2 wastewater monitoring data has largely involved comparison of viral concentrations in wastewater to COVID-19 case counts based on clinical 463 464 testing.³⁵ Because the accuracy of confirmed case rates as a measure of the number of infected individuals is dependent on COVID-19 testing rates, this comparison must be made with a 465 466 consideration of clinical testing biases. Moreover, if multiple clinical data types are available, one must determine which is most appropriate for comparison to wastewater data. The analysis 467 468 applied herein utilized a data set containing 7-day averages of new COVID-19 cases based on testing in each approximated sewershed area. Uncertainties surrounding such clinical testing data 469 470 include (1) whether there were regional biases in testing results (Figure S.2), potentially due to testing disparities;¹¹ (2) whether testing rates were adequate and what constitutes adequate 471 testing; and (3) how long before specimen collection infected individuals contracted COVID-19 472 and started shedding the virus. Others have reported correlations of wastewater data with 473 474 COVID-19 surveillance data sets other than clinical case rates, such as clinical test positivity or 475 hospitalization rates.² Hospital admissions data, although not without its own biases,³⁶ may be an 476 alternative epidemiological metric to compare to or to validate wastewater monitoring data if 477 significant inadequacies in clinical testing are suspected. While hospitalization data at the 478 MODZCTA level were not publicly available for NYC, visual comparison at the borough level indicates that trends in daily hospitalizations generally reflect trends in case rates for sewersheds 479 480 within each borough (Figure S.3). The limitations of clinical testing are in fact a major driver for the application of WBE, which aims to provide community-level information free from clinical 481 482 testing bias.^{37–39} Continued population-level monitoring from wastewater data could become 483 increasingly useful in areas where clinical testing rates decline or resources for clinical testing 484 are limited.

485

486 Linear regressions for the combined data set are presented in Figure 3 with data collected on 487 dates with over 10% positive COVID-19 testing rates removed. Removing data associated with 488 potentially inadequate testing from the combined data set did not significantly change the 489 regression (Analysis of Covariance, p > 0.05) compared to the full data set without filtering 490 (Figure S.5). After the peak in COVID-19 cases in NYC in January 2021, there was a decline in 491 cases across all sewersheds. To assess whether the relationship between SARS-CoV-2 loads in 492 wastewater and new clinical COVID-19 cases was significantly different during the period of 493 declining cases from that during the period when cases were increasing, we compared separate 494 linear regressions for the data associated with the rise in case rates (data prior to January 2021) 495 and the decline in case rates (data after January 2021). No significant differences were found 496 between the slopes of the linear regression lines determined using the full combined data set and 497 the data separated based on time period.

498

The slope of the linear regression line for the full combined data set was found to be 0.6, indicating that a 1 log₁₀ change in the number of N1 GC/day corresponded to a 0.6 log₁₀ change in the number of new laboratory-confirmed COVID-19 cases/day in a sewershed. Metrics such

as these are derived from relative changes in viral load, and therefore do not require absolute

503 guantification of viral concentrations in wastewater, allowing for comparison to other studies and

alleviating challenges related to absolute quantification of standard curves. However, this metric

- 504 alleviating challenges related to absolute quantification of standard curves. However, this metri
- 505 comparing SARS-CoV-2 loads and daily new COVID-19 cases has not been consistently
- reported in studies monitoring SARS-CoV-2 in influent wastewater. Harmonizing data analysis

strategies to include such a metric would improve efforts to compare results across different
locations. The slope of 0.6 observed herein is greater than that reported previously by Wolfe et
al. (slope = 0.24), who compared SARS-CoV-2 concentrations measured in primary wastewater
settled solids and COVID-19 incidence in seven publicly owned treatment works located across

- the United States, including one of the NYC facilities described in this work.³⁵ In addition to
- analyzing a different type of sample for SARS-CoV-2 concentrations (i.e., primary settled solids
- 513 versus influent wastewater), the analysis used by Wolfe et al. (2021) differed from that herein in
- 514 that they normalized measured SARS-CoV-2 concentrations in wastewater solids by
- 515 concentrations of pepper mild mottle virus (PMMoV). The differences in the slopes may be due
- to either of these factors, to variations in the relationship between SARS-CoV-2 wastewater
 loads and COVID-19 cases in different regions, or to a difference in the overall sensitivity of the
- 518 methodology applied by Wolfe et al.
- 519
- 520 At present, limitations regarding the accuracy of COVID-19 clinical testing data and
- 521 uncertainties related to SARS-CoV-2 measurements in wastewater--including SARS-CoV-2
- shedding rates and RNA stability in different sewersheds--preclude development and validation
- 523 of a universal, quantitative model to predict disease incidence based on viral RNA concentrations
- 524 in wastewater. Ongoing research continues to expand our understanding of critical model
- parameters and factors contributing to uncertainty, owing particularly to SARS-CoV-2
 monitoring work completed at smaller scales (e.g., building-level),⁴⁰ from which information
- about the contributing population can be obtained more easily than from larger sewersheds. An
- 527 about the contributing population can be obtained more easily than nonnaiger sewersheds. An
 528 attempt to quantify COVID-19 case rates in NYC's sewersheds based on wastewater data at this
- 529 time would be inaccurate, and is not currently recommended for application in the realm of
- 530 public health.⁴¹ However, based on our analysis and others, there is utility in using wastewater
- 531 data to monitor trends in COVID-19 incidence.
- 532
- 533 Estimated case rates associated with method LOD
- The utility of SARS-CoV-2 wastewater data depends on whether virions are present in
 wastewater at detectable concentrations (i.e., above the LOD and LOQ). It is therefore useful to
 approximate the minimum number of contributing COVID-19 cases per day required for
 detection of the SARS-CoV-2 N1 gene target in wastewater using the methodology described
 here. When estimated using individual, sewershed-specific linear regressions (Figure 2), the
- 539 minimum new COVID-19 case rate that corresponds to the method LOD varied for each
- sewershed, ranging between 2 and 8 cases/day/100,000 people (Table S.4). Minimum detectable
- 541 case rates were also estimated for each sewershed using the linear regression from the combined
- data set and the average daily influent flow rates for each WRRF during the study period. These
 estimates fell within the same range as those derived from sewershed-specific linear regressions
- 543 estimates fell within the same range as those of544 (Table S.4).
 - 545
 - 546 The minimum detectable case rate estimates presented here should be taken as order-of-
 - 547 magnitude approximations rather than absolute quantities, especially considering the varying
 - 548 strength of the linear relationships between data for certain sewersheds (e.g., data sets for Coney
 - 549 Island, Bowery Bay, Oakwood Beach, and Port Richmond WRRFs had Pearson correlation
 - 550 coefficients below 0.5). Furthermore, these findings hold only for the specific SARS-CoV-2
 - quantification methodology applied herein, and may not be transferable to locations with
 - 552 different per capita wastewater flow rates, even if testing rates and case rates are similar to those

described here. The estimates may also be limited by the assumption that the dominant source of
the SARS-CoV-2 viral load in the wastewater is from recent cases as opposed to prolonged fecal
shedding, which is consistent with assumptions made in previous studies.^{35,42} Furthermore,
variability in virus shedding rates were not considered for the simple linear models in our study.
The relationships found are also limited by the accuracy of clinical testing data, as discussed
above.

- 559 560 As COVID-19 cases declined in NYC in the spring and early summer of 2021, the estimated minimum detectable COVID-19 case rates were reached in most sewersheds by May and June 561 562 2021. As such, we expected that SARS-CoV-2 viral loads in wastewater would have decreased 563 to below the LOQ and LOD at this time. However, viral RNA was still detectable in influent 564 wastewater collected from all sewersheds in mid June 2021 (Figure 4). While this discrepancy may be explained by the limitations described above, it may also be due to decreasing COVID-565 19 testing rates, which could result in reduced diagnosis of individuals with asymptomatic 566 infections, who are less likely to seek out COVID-19 tests. The average COVID-19 testing rate 567 in NYC during the period from May 2, 2021 to June 8, 2021 decreased 30% from the average in 568 569 January 2021. Additionally, widespread vaccination of adults in New York may have resulted in 570 asymptomatic and mild infections that were not diagnosed. While individuals with asymptomatic 571 SARS-CoV-2 infections may not be captured by clinical testing, viral shedding by asymptomatic 572 individuals would still contribute to the viral load in wastewater, given that SARS-CoV-2 has been detected in fecal samples associated with asymptomatic or mild cases of COVID-19.43-45 573 574 Viral loads may have also been elevated in wastewater because of prolonged fecal shedding of 575 the virus. Finally, it is possible that the linear relationship found in this work does not hold at low 576 SARS-CoV-2 infection levels as the study period used for statistical analysis included only case 577 rates above the minimum detectable case rates estimated for each sewershed.
- 578
- 579

580 The estimated minimum numbers of COVID-19 cases required before SARS-CoV-2 can be

detected in wastewater from NYC sewersheds are associated with considerable disease incidence
 that may be captured if some degree of clinical testing continues. Nonetheless, these estimates
 could aid public health agencies in understanding what COVID-19 incidence to expect if SARS CoV-2 loads measured in wastewater influent cross the threshold from being below the detection
 limit to being detected. Improvements to analytical methods that lower the LOD^{46–48} would

586 expand the utility of WBE in indicating low levels of disease incidence.

587

588 Conclusion

589

- 590 Critical choices made at the beginning of the development of NYC's SARS-CoV-2 wastewater 591 monitoring program proved beneficial for the long-term wastewater monitoring goals for NYC, 592 and highlight strategies that may be useful for agencies interested in implementing wastewater 593 monitoring programs for emerging pathogens. First, collaborating parties--including academic 594 partners and NYC DEP personnel--worked together to develop a monitoring program centered 595 around NYC DEP's priorities. Second, sample analysis was conducted in a NYC DEP 596 microbiology laboratory, which allowed the program to take advantage of existing equipment,
- 597 expertise, protocols, and resources related to wastewater analysis, as well as existing wastewater
- 598 sampling and transport protocols and infrastructure. Doing so expedited the initiation of the

599 wastewater monitoring program and supported virus analysis capacity building within the NYC 600 DEP. With this structure, routine monitoring began in parallel with training and continued method optimization. Consequently, protocol adjustments responded to practical challenges as 601 602 well as technical ones, taking into account laboratory infrastructure and equipment that would 603 ultimately be used for the ongoing monitoring program. This also made for a rich training 604 experience, in which analysts shared insights from hands-on experience, contributed to workflow 605 decisions, and were exposed to the empirical reasoning behind methodological choices. Direct 606 communication between wastewater treatment facility operators and laboratory personnel 607 maximized use of the NYC DEP's extensive knowledge base and data, which aided in troubleshooting.

608 609

610 As WBE programs for wastewater-related viruses evolve to meet future challenges, continued

- 611 research is needed to better understand the mechanisms by which virus concentration, extraction,
- and quantification methods work, and the factors that influence the efficiency of each step; this
- 613 knowledge can subsequently inform method optimization, standardization, and the accounting of
- 614 methodological uncertainty. Since the implementation of the SARS-CoV-2 wastewater
- 615 monitoring program in NYC, several studies have begun to evaluate and compare different
- sample processing strategies, including one interlaboratory study which included the
- 617 methodology used herein. $^{48-50}$ A clear characterization of the limitations and benefits of
- 618 methodological choices for virus enumeration is critical for not only assessing previously
- collected data but also comparing results between WBE programs implemented by different
 parties, and informing future efforts in the WBE field. For example, varied priorities, resources,
- and expertise in different WBE programs may foster the continued use of many different
- 622 methods rather than the adoption of one universal method. Additionally, poorly characterized
- 623 variability in WBE data stands in the way of the critical goal of relating viral loads in wastewater
- 624 to disease dynamics. Clear characterization of uncertainties related to analytical methodologies
- 625 would therefore facilitate interpretation of wastewater data by public health agencies.⁵¹
- 626 Nonetheless, results from NYC's monitoring program show that relative trends in SARS-CoV-2
- 627 loads in wastewater can be evaluated and associated with trends in clinical testing data, and
- therefore can potentially contribute to situational awareness of disease incidence in large urbansewersheds.
- 630

631 Conflicts of Interest

- 632 There are no conflicts of interest to declare.
- 633

634 Acknowledgements

635

Funding for this work was provided by the New York City Department of EnvironmentalProtection and the Alfred P. Sloan Foundation.

638

639 An extensive team at the NYC DEP made this monitoring program possible, including Samantha

640 MacBride, Peter Williamsen, Gina Behnke, Jasmin Torres, and Jorge Villacis; members of the

641 NYC DEP Microbiology Lab, including William Kelly, Naudet Joasil, Patrick Hoyes, Donnovan

Johnson, Manzura Kopusov, Oren Sachs, and Samantha Cruickshank; the NYC DEP

transportation team, including Lateef Franklin, Samuel Young, and John Congemi; and Abeba

- 644 Negatu, Patrick Jagessar, Max Verastegui and their process control laboratory teams at NYC645 DEP.
- 646

647 Several researchers at CUNY provided support and assistance for protocol development,

optimization, and training, including Sherin Kannoly, Kaung Myat "Zach" San, Kristen Cheung,
 Anna Gao, Michelle Markman, Nanami Kubota, and Irene Hoxie.

650

651 We thank Alexandria Boehm (Stanford University) and Sandra Mclellan (University of

652 Wisconsin-Milwaukee) for their support and guidance during program development. We also

acknowledge the many insights gained from the interactions through the NSF Research

654 Coordination Network (RCN) on Wastewater Surveillance for SARS-CoV-2.

655

A script automating the download of New York City's publicly available COVID-19 clinical

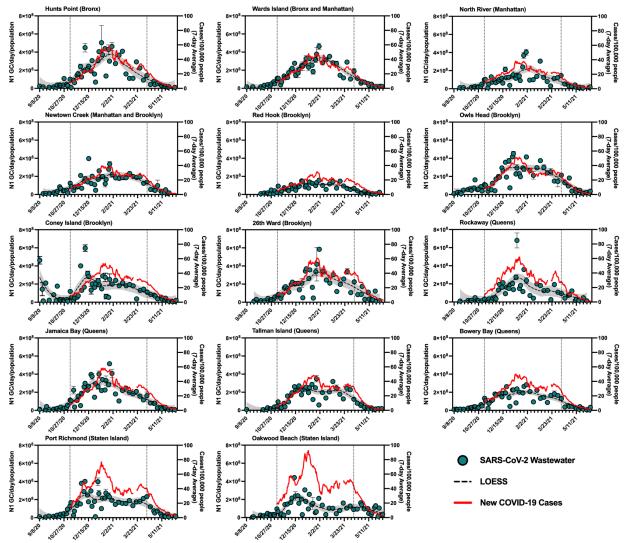
657 testing data was generously provided by Charlie Mydlarz (NYU Center for Urban Science and

658 Progress).

659 Figures

660 Note that the N1 concentrations reported in the following figures may be updated in future

- versions of this work to reflect the quantified concentration of the RT-qPCR standard, which is
- 662 currently being quantified. These updates should not change observed trends reported here, as
- 663 described in the main text.



664

Figure 1. Summary of SARS-CoV-2 wastewater data for New York City's 14 sewersheds. 665 Data from September 8, 2020 to June 8, 2021 is shown, with the period for which statistical 666 analysis was conducted (November 8, 2020 to April 11, 2021) bounded by vertical dotted lines. 667 Primary (left) y-axis, blue circles: Influent SARS-CoV-2 viral loads normalized by sewershed 668 populations. Error bars indicate standard deviations from triplicate RT-qPCR reactions as well as 669 670 standard deviations of duplicate samples, where applicable. Dashed black lines represent LOESS curve fits (span = 0.4), with the 95% confidence intervals shaded in grey. Secondary (right) y-671 axis, red line: 7-day average of new COVID-19 cases/day/100,000 people in the previous 7 days 672 normalized using MODZCTA-level population estimates from the NYC DOHMH's NYC 673 Coronavirus Disease 2019 (COVID-19) Data.¹⁸ Normalization by population was used for visual 674 675 comparison across different sewersheds only and was not used for statistical analysis.

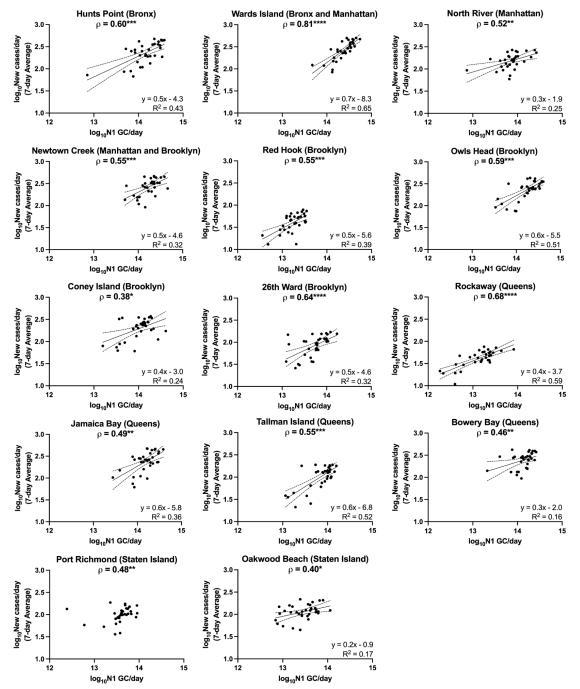
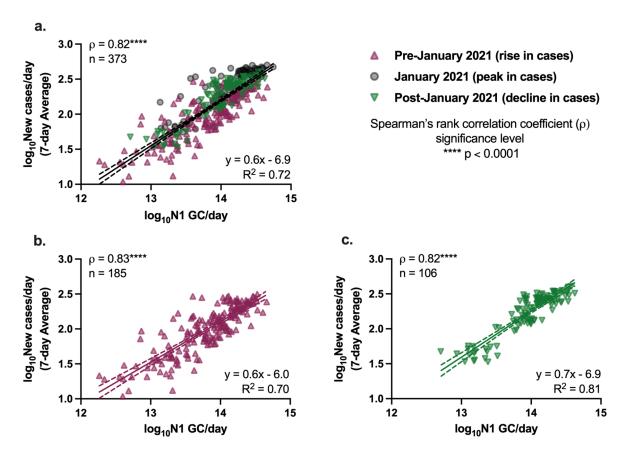
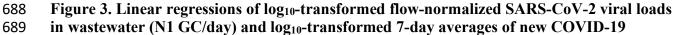




Figure 2. Linear regressions of log10-transformed SARS-CoV-2 viral loads in wastewater 678 (N1 GC/day) and log₁₀-transformed 7-day averages of new COVID-19 cases/day for each 679 sewershed in New York City. Linear regressions (solid lines) and associated 95% confidence 680 681 intervals (dashed lines) are shown along with goodness of fit R² values for those data sets with significantly non-zero slopes. Note that linear regression for Port Richmond has been excluded 682 as the slope was not significantly non-zero (see SI). The Spearman's rank correlation coefficient 683 (ρ) between N1 GC/day and new COVID-19 cases/day is shown at the top of each sewershed 684 plot, with significance levels indicated (*p < 0.05, **p < 0.01, ***p < 0.001, ***p <685 686 0.0001).



687



690 cases/day for (a) the combined data set, (b) data from the combined data set associated with

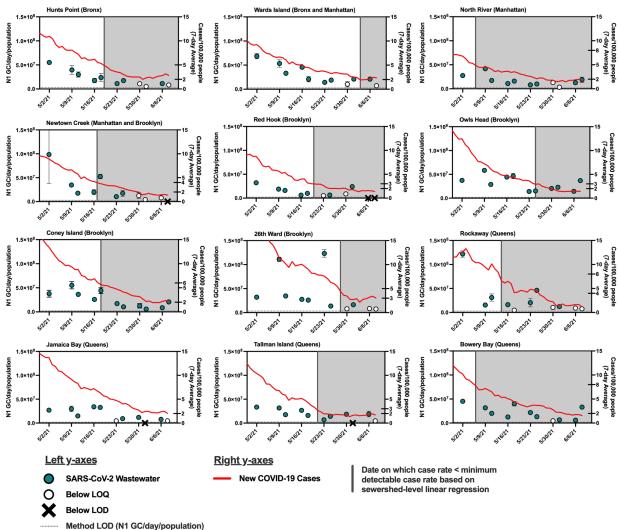
a rise in cases, and (c) data from the combined data set associated with a decline in cases.

Data associated with potentially inadequate testing (i.e., over 10% positive tests) are not included

in this analysis. Linear regressions (solid lines) and associated 95% confidence intervals (dashed
 lines) are shown along with goodness of fit R² values and Spearman's rank correlation

694 lines) are shown along with goodness of fit R^2 values and Spearman's rank correlation 695 coefficients (ρ) between N1 GC/day and new COVID-19 cases/day.

696



697 Figure 4. SARS-CoV-2 wastewater data and COVID-19 case data from May 2, 2021 to 698 June 8, 2021. The date on which the case rate first fell below the estimated minimum detectable 699 case rate (based on the sewershed-level linear regression) is indicated with a solid vertical line 700 for each sewershed. Shaded regions indicate the time period during which case rates were below 701 702 the estimated minimum detectable case rate. Primary (left) y-axis, blue circles: Influent SARS-CoV-2 viral loads normalized by sewershed populations. Error bars indicate standard deviations 703 704 from triplicate RT-qPCR reactions as well as standard deviations of duplicate samples, where

- applicable. Open circles represent N1 concentrations below the limit of quantification (LOQ). 705
- Samples below the limit of detection (LOD, shown with a horizontal dotted line) are denoted 706
- with an "X." Secondary (right) y-axis, red line: 7-day average of new COVID-19 707
- 708 cases/day/100,000 people in the previous 7 days. Estimated minimum detectable case rates (new
- 709 cases/day/100,000) needed to detect SARS-CoV-2 in wastewater, based on linear regressions
- derived from sewershed-level data and the combined data set, are indicated with tick marks 710
- 711 across the y-axes.
- 712
- 713
- 714

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- 884 September 2021 Emerging Infectious Diseases journal CDC, 2021,
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886

Appendix 3

Detection of Mutations Associated with Variants of Concern Via High Throughput 1

- Sequencing of SARS-CoV-2 Isolated from NYC Wastewater 2
- 3
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- 20

21 ABSTRACT (186 words)

Monitoring SARS-CoV-2 genetic diversity is strongly indicated because diversifying 22 23 selection may lead to the emergence of novel variants resistant to naturally acquired or 24 vaccine-induced immunity. To date, most data on SARS-CoV-2 genetic diversity has 25 come from the sequencing of clinical samples, but such studies may suffer limitations 26 due to costs and throughput. Wastewater-based epidemiology may provide an alternative and complementary approach for monitoring communities for novel variants. 27 Given that SARS-CoV-2 can infect the cells of the human gut and is found in high 28 concentrations in feces, wastewater may be a valuable source of SARS-CoV-2 RNA, 29

- 30 which can be deep sequenced to provide information on the circulating variants in a
- community. Here we describe a safe, affordable protocol for the sequencing of SARS-
- 32 CoV-2 RNA using high-throughput Illumina sequencing technology. Our targeted
- 33 sequencing approach revealed the presence of mutations associated with several
- Variants of Concern at appreciable frequencies. Our work demonstrates that
- 35 wastewater-based SARS-CoV-2 sequencing can inform surveillance efforts monitoring
- the community spread of SARS-CoV-2 Variants of Concern and detect the appearance
- of novel emerging variants more cheaply, safely, and efficiently than the sequencing of
- 38 individual clinical samples.

39 **IMPORTANCE (140 words)**

- 40 The SARS-CoV-2 pandemic has caused millions of deaths around the world as
- 41 countries struggle to contain infections. The pandemic will not end until herd immunity is
- reached, that is, when most of the population has either recovered from SARS-CoV-2
- 43 infection or is vaccinated against SARS-CoV-2. However, the emergence of new SARS-
- 44 CoV-2 variants of concern threatens to erase gains. Emerging new variants may re-
- 45 infect persons who have recovered from COVID-19 or may evade vaccine-induced
- immunity. However, scaling up SARS-CoV-2 genetic sequencing to monitor Variants of
- 47 Concern in communities around the world is challenging. Wastewater-based
- 48 sequencing of SARS-CoV-2 RNA can be used to monitor the presence of emerging
- variants in large communities to enact control measures to minimize the spread of these
- variants. We describe here the identification of alleles associated with several variants
- of concern in wastewater obtained from NYC watersheds.
- 52 **KEYWORDS:** coronavirus, environmental microbiology, Illumina sequencing,
- 53 metagenomics, NGS, sewage, virus surveillance, Variants of Concern, wastewater-
- 54 based epidemiology

55 INTRODUCTION

- 56 The emergence of novel SARS-CoV-2 Variants of Concern, including B.1.1.7 from the
- 57 United Kingdom and B.1.351 from South Africa, has provoked intense speculation about
- the future of the pandemic (1-3). Early studies suggest that these new variants may be
- 59 more transmissible (4-6). Even more concerning are reports of decreased antibody-
- 60 mediated neutralization of these variants (7-9). Regardless of the biological attributes of

61 these novel variants, it is clear that behavioral interventions, public health measures,

- vaccinations, and reduced numbers of susceptible individuals will impose strong
- 63 diversifying selection on SARS-CoV-2 to enhance transmission and/or evade host
- 64 immunity (10). We should anticipate that the continued evolution of SARS-CoV-2 may
- result in variants that evade natural or vaccine-mediated immunity. As such, intensive
- 66 monitoring of SARS-CoV-2 genetic diversity and evolution is vital to rapidly identify
- 67 Variants of Concern as they emerge.

68 Currently, most SARS-CoV-2 genetic surveillance is conducted via the genome

- 69 sequencing of viral RNA obtained from clinical specimens. While occurring at a much
- greater rate and volume than previous epidemics, the sequencing of clinical specimens
- is limited by cost, coverage, quality, and throughput concerns. In developed countries,
- these issues are not readily apparent, but sequencing efforts in underdeveloped
- countries has been more restricted (11). Another disadvantage of focusing on clinical
- strains stems from the large number of asymptomatic or mildly symptomatic infections
- 75 (12). SARS-CoV-2 sequencing efforts will suffer biases if genomic information is more
- ⁷⁶ frequently obtained from seriously ill patients, rather than from asymptomatic patients,
- and those with mild symptoms who choose to follow the CDC's advice and convalesce
- at home. Wastewater-based epidemiology may provide an alternative and
- complementary approach to provide more representative SARS-CoV-2 genetic data at
 lower costs and higher throughput.
- Given that SARS-CoV-2 has been detected in fecal samples (13, 14), and subsequently
- in wastewater, wastewater is being monitored in communities around the world to
- determine SARS-CoV-2 prevalence in communities (15-17). Furthermore, isolation of
- 84 SARS-CoV-2 RNA from wastewater coupled with high-throughput deep sequencing
- provides an almost unlimited source of unbiased viral sequences, which can be used to
- 86 monitor frequencies of Variants of Concern in populations (18-20). We have focused on
- 87 the use of targeted sequencing of the spike genomic region known to encode Variants
- 88 of Concern. Our approach, while limited to a specific region of the genome, is
- 89 affordable, rapid and generates sufficient coverage to quantify known variants and to
- 90 identify possible emerging ones.
- 91 Our team, in conjunction with the New York City Department of Environmental
- 92 Protection, has been monitoring the genetic signal of SARS-CoV-2 in the wastewater of
- all 14 wastewater treatment plants in NYC, an area that encompasses a population of
- 8,419,000 persons, since June 2020. We developed and optimized a protocol for safe,
- ⁹⁵ cost-effective, and repeatable quantitation of SARS-CoV-2 copy number by RT-qPCR
- 96 (21). Our protocol performed strongly in a large-scale, nationwide comparative study of
- 97 the reproducibility and sensitivity of 36 methods of quantifying SARS-CoV-2 in
- 98 wastewater (22). Our protocol is identified as 4S.1(H) in Table 3. We further extended
- 99 the utility of our protocol by deep sequencing SARS-CoV-2 RNA isolated from
- 100 wastewater samples. Here we report presence of alleles associated with different
- 101 Variants of Concern at appreciable frequencies. Our findings provide support for recent

- 102 observations of increasing frequencies of New York Variant of Interest B.1.526 in
- clinical samples (23, 24), as well as the presence of Variants of Concern from the
- 104 United Kingdom, California, South Africa and Brazil (25). Furthermore, our results
- demonstrate the utility of wastewater-based epidemiology for the timely identification of
- novel variants of concern arising in communities.

107 **RESULTS AND DISCUSSION**

108 Targeted sequencing is a viable approach for identifying SARS-C0V-2 mutations.

- 109 We generated cDNA from NYC wastewater samples that exhibited RT-qPCR Cts values
- ranging from 28 to 24 Cts corresponding to 26,443 and 1,423,339 N1 copies/L,
- respectively. Using this cDNA as a template, we PCR amplified a region of the receptor
- binding domain (RBD) of the SARS-CoV-2 Spike gene, spanning amino acid residues
- 113 P410 to L513, which encompasses mutations that are found in several known Variants
- of Concern. A total of 420 single nucleotide variants were identified in the 45 samples
- sequenced (Supplementary Table 1). Coverage ranged from 1,037x 118,737x with a
- mean of 23,586x (Supplementary Table 1). Across all samples, we identified 75 unique
- 117 mutations resulting in amino acid substitutions, 20 unique synonymous mutations, and
- 118 18 deletions resulting in a frameshift, in the 332 bp region targeted (Supplementary 119 Table 1).

120 Mutations associated with Variants of Concern are present in NYC wastewater.

- 121 The five mutations found at highest frequencies, both in terms of frequency of reads
- within samples and found in the most samples, were L452R, E484K, N501Y, S494P,
- and S477N. All five mutations are associated with known Variants of Concern (Fig. 1;
- 124 Supplementary Table 2). On Jan 31st, we sequenced samples from two wastewater
- treatment plants in NYC and identified reads containing mutations L452R, S477N,
- 126 E484K, S494P and N501Y in both. On February 28th and March 14th samples from all
- 127 14 wastewater treatment plants in NYC were sequenced, revealing the presence of a
- high proportion of reads containing mutations L452R, S477N, E484K, S494P and
- 129 N501Y (Fig. 1). Mutation L452R is unique to Pango lineage Variants of Concern
- B.1.427 and B.1.429, which were first observed in California (25, 26). Mutation S477N is
- only found in New York Variant of Interest B.1.526 (23-25, 27). Mutation E484K has
- been reported in Variants of Concern B.1.1.7 from the United Kingdom, P.1 and P.2
- from Brazil, and B.1.351 from South Africa, and B.1.525 and B.1.526 from New York
- (25). Mutation S494P is only found in Variant of Concern B.1.1.7 from the United
- 135 Kingdom (25). Mutation N501Y is found in Variants of Concern B.1.1.7 from the United
- Kingdom, P.1 from Brazil, and B.1.351 from South Africa (25).
- 137 The finding that unique mutations associated with different Variants of Concern in our
- pooled sequencing assay suggests the circulation of these variants in NYC. A caveat
- 139 with our approach, however, is that we cannot conclusively identify the presence of a
- 140 Variant of Concern since our sequencing assay targets only a region of the receptor
- binding domain, and some significant mutations are outside the sequenced region.
- 142 Furthermore, additional mutations occurring in the primer binding region may allow

- some mutations to go undetected because their DNA could not be amplified. We are
- expanding our targeted sequencing approach to include additional regions of interest to
- 145 minimize the chance of missing important variants. Additionally, we intend to generate
- 146 cDNA with random hexamers, and to incorporate a level of degeneracy in the
- sequencing primers to increase the breadth of our targeted sequencing.
- ¹⁴⁸ Our most recent data from March 14th suggests a slight decrease in the prevalence of
- the E484K variant, but we cannot draw firm conclusions due to the nature of our
- sequencing assay, which relies on the collective sequencing of a large pool of
- individuals. Nevertheless, our frequency data agrees with that recently observed in
- human clinical samples from NYC (23, 24, 27). We intend to supplement our targeted
- 153 sequencing approach with whole genome amplicon sequencing in the future.
- 154 We believe that our approach offers a viable alternative to whole genome sequencing
- 155 for the detection of known variants and can be rapidly deployed to detect additional
- emerging variants of concern. Importantly as a cost saving measure, labs can generate
- 157 the libraries themselves and outsource the sequencing component to companies/core
- 158 facilities if they lack access to a sequencer, generally with a short turnaround time.

159 MATERIALS AND METHODS

- 160 Wastewater Sample Processing and RNA Extraction. Wastewater was collected
- 161 from 14 NYC wastewater treatment plants and RNA isolated according to our previously
- published protocol (dx.doi.org/10.17504/protocols.io.brr6m59e) (21). Control SARS-
- 163 CoV-2 synthetic RNA was purchased from Twist Bioscience (#102019).
- Briefly, 250 mL from 24-hr composite raw sewage samples were obtained from NYC
- wastewater treatment plants (WWTPs) and centrifuged at 5,000 x g for 10 min at 4°C to
- pellet solids. 40 mL of supernatant was passed through a 0.22 µM filter. Filtrate was
- 167 stored at 4°C for 24 hrs after adding 0.9 g sodium chloride and 4.0 g PEG 8000 (Fisher)
- then centrifuged at 12,000 x g for 120 minutes at 4 °C to pellet precipitate. The pellet
- 169 was resuspended in 1.5 mL TRIzol (Fisher), and RNA was purified according to the
- 170 manufacturer's instructions.
- 171 **Targeted PCR.** Our target for sequencing was a 332 bp region of the Receptor Binding
- Domain (RBD) of the spike protein spanning amino acid residues P420 to L513.
- 173 Mutations in this region are of critical importance as they might help the variants evade
- current antibody treatments and vaccines. RNA isolated from wastewater was used to
- 175 generate cDNA using ProtoScript® II Reverse Transcriptase (New England Biolabs).
- 176 The RNA was incubated with an RBD specific primer (ccagatgattttacaggctgcg) and
- dNTPs (0.5 mM final concentration) at 65°C for 5 minutes and placed on ice. The RT
- buffer, DTT (0.01 M final concentration), and the RT were added to the same tube and
- incubated at 42°C for 2 hours followed by 20 minutes at 65°C to inactivate the enzyme.
- The RBD region was amplified using Q5® High-Fidelity DNA Polymerase using the
 forward primer 5' -
- 182 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGccagatgattttacaggctgcg-3' and

- 183 reverse primer 5'-
- 184 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGgaaagtactactactctgtatggttgg-3',
- 185 which incorporate Illumina adaptors. PCR performed as follows: 98°C for 30 seconds,
- followed by 40 cycles of 98°C 5 seconds, 53°C for 15 seconds and 65°C for 1 minute and
- 187 a final extension at 65°C for 1 minute.

Targeted Sequencing. The RBD amplicons were purified using AMPure XP beads

- 189 (Beckman Coulter). Index PCR was performed using the Nextera DNA CD Indexes kit
- 190 (Illumina) with 2X KAPA HiFi HotStart ReadyMix (Roche), and indexed PCR products
- ¹⁹¹ purified using AMPure beads. The indexed libraries were quantified using the Qubit 3.0
- and Qubit dsDNA HS Assay Kit and diluted in 10 mM Tris-HCl to a final concentration of
- approximately 0.3 ng/ μ L (1 nM). The libraries were pooled together and diluted to a final
- concentration of 50 pM. Before sequencing on an Illumina iSeq100, a 10% spike-in of
- 195 50 pM PhiX control v3 (Illumina) was added to the pooled library.
- 196 **Bioinformatics.** Sequencing data was uploaded to the BaseSpace Sequence Hub, and
- the reads demultiplexed using a FASTQ generation script. Reads were processed using
- the published Geneious workflows for preprocessing of NGS reads and assembly of
- 199 SARS-CoV-2 amplicons (https://help.geneious.com/hc/en-us/articles/360045070991-
- 200 Assembly-of-SARS-CoV-2-genomes-from-tiled-amplicon-Illumina-sequencing-using-
- 201 Geneious-Prime and https://help.geneious.com/hc/en-us/articles/360044626852-Best-
- 202 practice-for-preprocessing-NGS-reads-in-Geneious-Prime). Paired reads were trimmed,
- and the adapter sequences removed with the BBDuk plugin. Trimmed reads were
- merged and aligned to the SARS-CoV-2 reference genome MN908947. Variants were
- called using the Annotate and Predict Find Variations/SNPs in Geneious and verified by
- using the V-PIPE SARS-CoV-2 application (<u>https://cbg-ethz.github.io/V-pipe/sars-cov-</u>
- 207 <u>2/</u>)(28).

208 Data Availability

- 209 Raw sequencing reads are available in NCBI's Sequence Read Archive (SRA) under
- 210 accession # PRJNA715712.

211 ACKNOWLEDGEMENTS

- The research described herein would not be possible if not for the assistance and
- support of a wide-range of organizations and individuals that came together to address
- the shared calamity that is the COVID-19 pandemic. We thank Gina Behnke, Esmeraldo
- 215 Castro, Francoise Chauvin, Alexander Clare, Pilar Domingo-Calap, Pam Elardo, Raul
- Gonzalez, Crystal Hepp, Catherine Hoar, Dimitrios Katehis, William Kelly, Samantha
- 217 McBride, Hope McGibbon, Hilary Millar, Samantha Patinella, Krish Ramalingam, Andrea
- 218 Silverman, Jasmin Torres, Arvind Varsani, Peter Williamsen for support, advice,
- discussions, and feedback. This work was funded in part by the New York City
- 220 Department of Environmental Protection. The Water Research Foundation, the NSF
- 221 Research Coordination Network for Wastewater Surveillance for SARS-CoV-2 and

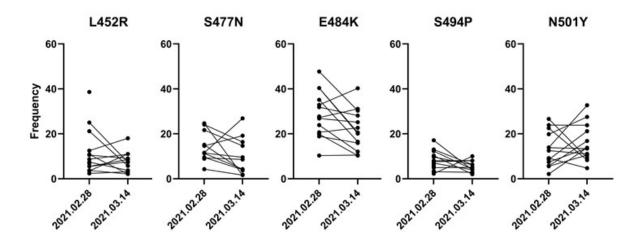
- 222 Qiagen Inc. provided resources, materials and supplies, technical support, and
- community support.

224 FIGURE LEGEND

- Figure 1. Frequencies of reads associated with five selected mutations associated with
- 226 SARS-CoV-2 Variants of Concern from wastewater obtained from 14 NYC wastewater
- treatment plants on two separate dates.

228

229 Figure 1





231

232

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Tracking Cryptic SARS-CoV-2 Lineages Detected in NYC Wastewater

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Abstract

Tracking SARS-CoV-2 genetic diversity is strongly indicated because diversifying selection may lead to the emergence of novel variants resistant to naturally acquired or vaccine-induced immunity. To monitor New York City (NYC) for the presence of novel variants, we amplified regions of the SARS-CoV-2 Spike protein gene from RNA acquired from all 14 NYC wastewater treatment plants (WWTPs) and ascertained the diversity of lineages from these samples using high throughput sequencing. Here we report the detection and increasing frequencies of novel SARS-CoV-2 lineages not recognized in GISAID's EpiCoV database. These lineages contain mutations rarely observed in clinical samples, including Q493K, Q498Y, H519N and T572N. Many of these mutations were found to expand the tropism of SARS-CoV-2 pseudoviruses by allowing infection of cells expressing the human, mouse, or rat ACE2 receptor. In addition, pseudoviruses containing the Spike amino acid sequence of these lineages were found to be resistant to many different classes of RBD binding neutralizing monoclonal antibodies. We offer several hypotheses for the anomalous presence of these mutations, including the possibility of an animal reservoir. Although wastewater sampling cannot provide direct inference of SARS-CoV-2 clinical sequences, our research revealed several lineages that could be relevant to public health and they would not have been discovered if not for wastewater surveillance.

Main

SARS-CoV-2 is shed in feces and can be detected in wastewater in proportion to caseloads in sewersheds^{1,2}. Since January of 2021, we sequenced SARS-CoV-2 RNA isolated from all 14 NYC WWTPs approximately twice per month³. Our targeted sequencing strategy entailed iSeq 100 and MiSeq sequencing of PCR-amplified regions of the SARS-CoV-2 Spike protein gene, particularly the receptor binding domain (RBD) (Fig. 1A). These regions span Spike protein amino acid residues 434 to 505 for iSeq amplicons and 412 to 579 for MiSeq amplicons. These regions contain loci that are significant in SARS-CoV-2 receptor tropism and immune evasion, and contain multiple polymorphisms found in many variants of concern (VOC)^{4,5}. Our analysis pipeline, which uses the tool SAM Refiner, allowed us to determine the frequency of each polymorphism and more importantly, elucidate which polymorphisms were derived from the same RNA sequence⁶.

Identification of Novel Sewershed-Specific Lineages

Using this approach, we were able to classify suites of mutations found in the RBD amplicons as consistent with Pango lineages B.1.1.7 (Alpha), B.1.351 (Beta), B.1.427/429 (Epsilon), B.1.526 (lota), B.1.617 (Delta and Kappa) and P.1 (Gamma). Importantly, the distributions and trends in viral lineages from wastewater were consistent with patient derived sequences from NYC (Fig. 1B)(Supplemental Table 1). For example, between February and April, wastewater surveillance and patient sequencing both revealed a notable increase in sequences assigned to the Alpha

lineage and a corresponding decrease in sequence that did not belong to any of the VOC lineages.

In addition to well-recognized lineages, three WWTPs, 3, 10, and 11, contained lineages with consistent, but not static, constellations of polymorphisms detected over several months that were inconsistent with lineages reported in the GISAID EpiCoV database (<u>https://www.gisaid.org/</u>)(Fig. 1C). Four of these lineages, designated WNY1, WNY2, WNY3, and WNY4, were selected for further study. Each of these lineages contained at least five polymorphisms; the most divergent was WNY4, which contained 16 amino acid changes in its RBD including the deletion of position 484.

Interestingly, all four novel lineages contained a polymorphism at position 498 (Q498H or Q498Y). As of July 16, 2021, there were only three US SARS-CoV-2 sequences in GISAID that contained the polymorphism Q498H, and none that contained Q498Y. However, both of these polymorphisms have been associated with host range expansion of SARS-CoV-2 into rodents^{7–9}, which are generally resistant to the parent SARS-CoV-2 lineage^{10–12}. Notably, as the concentration of SARS-CoV-2 genetic material from NYC wastewater decreased along with the decrease in SARS-CoV-2 patients, the fraction of the total sequences from these unknown lineages has proportionally increased. By May and June, these lineages often represented the majority of sequences recovered from some treatment facilities (Fig. 1C).

Are Cryptic Lineages Derived from Unsampled COVID-19 Infections?

The existence of these lineages may point to COVID-19 infections of human patients that are not being sampled through standard clinical sequencing efforts. The frequency of weekly confirmed cases in NYC that were sequenced ranged from 2.6% on January 31, 2021 to 12.9% on June 12, 2021¹³. It is not clear what strategies were employed to avoid non-random sampling of NYC COVID-19 infections, and the cryptic lineages may be derived from asymptomatic, vaccinated, immunosuppressed, pediatric, or chronically infected patients who are not being sampled in clinical settings. Infectious SARS-CoV-2 in such patients may linger in the gut after infections have resolved in the respiratory tract^{14–22}.

Alternatively, these lineages may be derived from physically distinct populations in the body. That is, perhaps viruses of these lineages predominantly replicate in gut epithelial cells and are not present in the nasopharynx such that standard swabbing techniques can recover sufficient quantities for sequencing. Another possibility is that genetically distinct virus populations can form in the gut and respiratory tract. Arguing against this latter possibility are sequencing data from patients showing that viruses extracted from feces was not genetically distinct from those extracted from the nasopharynx¹⁷. Finally, we speculate that perhaps these mutations are found in minority variants²³ that are unreported in consensus sequences uploaded to EpiCoV and other databases. While we were unable to assess wheth"er these sequences are derived from unsampled patients, we checked for minority variants in the raw reads of sequencing runs performed on samples obtained from NYC COVID-19 patients uploaded to NCBI's Sequence Read Archive (SRA). In addition, we searched SRA files

from other wastewater sequencing projects around the world. None of the WNY lineages were found.

Do Cryptic Lineages Indicate Presence of SARS-CoV-2 Animal Reservoirs?

Another hypothesis is that these lineages may point to the existence of SARS-CoV-2 animal reservoirs. To date, there have been a number of animal outbreaks, including in mink²⁴, lions and tigers²⁵, and cats and dogs²⁶. To gain insight into the host range of these lineages, synthetic DNA coding for the amino acid sequences for these four lineages were generated and introduced into a SARS-CoV-2 Spike expression construct for functional analysis (Fig. 2). All four of these lineages were found to be fully functional and produced transduction-competent lentiviral pseudoviruses with titers similar to the parent strain (D614G). To determine if these pseudoviruses displayed an expanded receptor tropism, stable cell lines expressing Human, Mouse, or Rat ACE2 were cultured with the pseudoviruses (Fig. 2). While the parent SARS-CoV-2 Spike pseudoviruses could only transduce cells with Human ACE2, all four of the WNY lineages could efficiently transduce cells with the Human, Mouse, and Rat ACE2. Because some patient-derived SARS-CoV-2 lineages such as Alpha have also gained the ability to infect rodent cells this observation cannot be taken as evidence that these lineages were derived from such a host. Nonetheless, the observation is consistent with the possibility that these lineages are derived from an animal host such as a rodent.

If such reservoirs exist, the animal host would need to meet several criteria. First, the host species would likely need to be present in the urban habitat in high densities such that epidemic transmission can be affected. Second, the number of susceptible animals present presumably must be high enough to sustain an epidemic for at least six months (i.e., the time period for which we observe these sequences). Finally, there must be a route for shed viruses to enter the sewershed.

We considered several mammal species known to inhabit NYC that may meet these criteria, including bats (several species), cats (*Felis catus*), dogs (*Canis familiaris*), grey squirrels (*Sciurus carolinensis*), mice (*Mus musculus* or *Peromyscus leucopus*), opossums (*Didelphis virginiana*), rabbits" (*Sylvilagus floridanus*), raccoons (*Procyon lotor*), rats (*Rattus norvegicus*), and skunks (*Mephitis mephitis*). To narrow our search, we reasoned that if viruses are being shed from one of these animals, then we should be able to detect rRNA from the animal in the sewershed as well.

Mammalian Species Detected in Wastewater

RNA extracted from wastewater and amplified with 12S and 16S rRNA primers (Supplementary Table 2) was deep sequenced. We were able to detect vertebrate rRNA in sewersheds where the cryptic lineages were found (Table 1). Several species, such as cow, pig, sheep, goat, and chicken, are not indigenous to NYC. These detects are likely derived from food consumption so are ruled out as possible hosts. Fish and duck rRNA detected likely stems from either food consumption or these animals may be inhabitants of bodies of water in the respective sewersheds. After non-indigenous

animals were removed, three remaining mammalian species were repeatedly detected: cats, dogs, and rats (Table 1).

Based on the consistent presence of their rRNA in NYC sewersheds (Table 1), cats, dogs, and rats are the most plausible animal reservoirs for SARS-CoV-2. Cats and dogs are known to be susceptible to SARS-CoV-2^{27,28}. Rodents are not permissive for infection by the canonical SARS-CoV-2 strain^{29,30}, but some variants allow infection of rodents³¹. A 2013 census estimated that there are 576,000 pet cats in NYC households³², but this estimate does not include stray cats. Extrapolating from a limited study conducted in 2017 implies a stray cat population of about 2,500 animals³³, but this number does not accord with the approximately 18,000 animals received annually by NYC Animal Care Centers³². There are currently 345,727 active dog licenses in NYC³⁴, but this figure is likely a significant underestimate and the true number may be at least double this figure. Despite these uncertainties, both cat and dog populations are dwarfed by the NYC rat population, which is estimated to number between 2-8 million animals³⁵.

Lineages Detected from Wastewater Are Resistant to Some Neutralizing Antibodies

In addition to polymorphisms from the WNY lineages that are known to affect viral tropism, many of the polymorphisms are also known to affect antibody evasion. In particular, the WNY polymorphisms at positions K417, N439, N440, K444, L452, N460, E484, Q493, S494, and N501 have all been reported to evade neutralization by particular antibodies^{4,36–39}. Most neutralizing antibodies against SARS-CoV-2 target the RBD of Spike, and most of these neutralizing antibodies are divided into 3 classes based on binding characteristics⁴⁰.

To test if the WNY lineages have gained resistance to neutralizing antibodies, we obtained three clinically approved neutralizing monoclonal antibodies representing these 3 classes, LY-CoV016 (etesevimab, Class 1), LY-CoV555 (bamlanivimab, Class 2), and REGN10987 (imdevimab, Class3), and tested their ability to neutralize the WNY lineages. All four of the WNY lineages displayed complete resistance to LY-CoV016, despite the parent lineage remaining potently sensitive to this antibody (Fig. 3). The WNY 1 and 2 remained at least partially sensitive to LY-CoV555 and REGN10987, but WNY 3 and 4 appeared to be completely resistant to all three neutralizing antibodies(Fig. 3). Finally, we tested the ability of plasma from fully vaccinated individuals (Pfizer) or patients previously infected with SARS-CoV-2 to neutralize WNY 3 and 4. All patients' plasma retained the capacity to neutralize these lineages (Fig. 3). However, previously infected patients had a greater reduction in ID50 (WT vs variant) than vaccinated patients and both were more affected by the WNY-4 variant than the WNY 3. It must be noted that neutralizing antibody activity from vaccinated individuals is not solely directed against the Spike RBD. Therefore, if the full Spike proteins from these lineages with the additional mutations they carry were tested, the neutralization capacity against these lineages is likely to be even further diminished. Thus, the characteristics of these variant lineages provide them the capacity to be an increased threat to human health.

Conclusions and Outlook

To date, most data on SARS-CoV-2 genetic diversity has come from the sequencing of clinical samples, but such studies may suffer limitations due to biases, costs and throughput. Here we demonstrate the circulation of several lineages of SARS-CoV-2 in the NYC metropolitan area that have been invisible to standard clinical surveillance. While the origins of these lineages have not been determined, we have demonstrated that these lineages have expanded receptor tropism which is consistent with expansion to an animal reservoir. Finally, we demonstrated that these lineages have gained significant resistance to patient-derived neutralized antibodies. Thus, these novel lineages could be relevant to public health and necessitate further study.

Materials and Methods

Wastewater Sample Processing and RNA Extraction

Wastewater was collected from the inflow at 14 NYC wastewater treatment plants and RNA isolated according to our previously published protocol². Briefly, 250 mL from 24-hr composite raw sewage samples obtained from NYC WWTPs were centrifuged at 5,000 x g for 10 min at 4°C to pellet solids. 40 mL of supernatant was passed through a 0.22 μ M filter (Millipore). Filtrate was stored at 4°C for 24 hrs after adding 0.9 g sodium chloride and 4.0 g PEG 8000 (Fisher Scientific) then centrifuged at 12,000 x g for 120 minutes at 4 °C to pellet the precipitate. The pellet was resuspended in 1.5 mL TRIzol (Fisher), and RNA was purified according to the manufacturer's instructions.

Targeted PCR

iSeq sequencing. RNA isolated from wastewater was used to generate cDNA using ProtoScript® II Reverse Transcriptase (New England Biolabs). The RNA was incubated with an RBD specific primer (ccagatgattttacaggctgcg) and dNTPs (0.5 mM final concentration) at 65°C for 5 minutes and placed on ice. The RT buffer, DTT (0.01 M final concentration), and the RT were added to the same tube and incubated at 42°C for 2 hours followed by 20 minutes at 65°C to inactivate the enzyme. The RBD region was amplified using Q5[®] High-Fidelity DNA Polymerase using primers that incorporate Illumina adaptors. PCR performed as follows: 98°C(0:30) + 40 cycles of [98°C(0:05) + 53°C(0:15) + 65°C(1:00)] x 40 cycles + 65°C (1:00).

The RBD amplicons were purified using AMPure XP beads (Beckman Coulter). Index PCR was performed using the Nextera DNA CD Indexes kit (Illumina) with 2X KAPA HiFi HotStart ReadyMix (Roche), and indexed PCR products purified using AMPure beads. The indexed libraries were quantified using the Qubit 3.0 and Qubit dsDNA HS Assay Kit and diluted in 10 mM Tris-HCl to a final concentration of approximately 0.3 ng/µL (1 nM). The libraries were pooled together and diluted to a final concentration of 50 pM. Before sequencing on an Illumina iSeq100, a 10% spike-in of 50 pM PhiX control v3 (Illumina) was added to the pooled library.

MiSeq sequencing. The primary RBD RT-PCR was performed using the Superscript IV One-Step RT-PCR System (Thermo Fisher Scientific). Primary RT-PCR amplification

was performed as follows: 25°C(2:00) + 50°C(20:00) + 95°C(2:00) + [95°C(0:15) + $55^{\circ}C(0:30) + 72^{\circ}C(1:00)$] x 25 cycles using the MiSeg primary PCR primers (Table 1). rRNA amplification used the same primary reaction conditions except containing 30 cycles using previously described 12s⁴¹ and 16s primers⁴². Secondary PCR (25 µl) was performed on RBD amplifications using 5 ul of the primary PCR as template with MiSeq nested gene specific primers containing 5' adapter sequences (Table 1) (0.5 µM each), dNTPs (100 µM each) and Q5 DNA polymerase (New England Biolabs). Secondary PCR amplification was performed as follows: 95°C(2:00) + [95°C(0:15) + 55°C(0:30) + 72°C(1:00)] x 20 cycles. A tertiary PCR (50 µl) was performed to add adapter sequences required for Illumina cluster generation with forward and reverse primers (0.2 µM each), dNTPs (200 µM each), and Phusion High-Fidelity DNA Polymerase (1U) (New England Biolabs). PCR amplification was performed as follows: 98°C(3:00) + [98°C(0:15) + 50°C(0:30) + 72°C(0:30)] x 7 cycles +72°C(7:00). Amplified product (10 µI) from each PCR reaction is combined and thoroughly mixed to make a single pool. Pooled amplicons were purified by addition of Axygen AxyPrep MagPCR Clean-up beads in a 1.0 ratio to purify final amplicons. The final amplicon library pool was evaluated using the Agilent Fragment Analyzer automated electrophoresis system, quantified using the Qubit HS dsDNA assay (Invitrogen), and diluted according to Illumina's standard protocol. The Illumina MiSeg instrument was used to generate paired-end 300 base pair length reads. Adapter sequences were trimmed from output sequences using cutadapt.

Wastewater rRNA Sequencing

cDNA from wastewater was also used to generate libraries using the primers indicated in Table 1.

Bioinformatics

iSeq reads were uploaded to the BaseSpace Sequence Hub, and demultiplexed using a FASTQ generation script. Reads were processed using the published Geneious workflows for preprocessing of NGS reads and assembly of SARS-CoV-2 amplicons⁴³. Paired reads were trimmed, and the adapter sequences removed with the BBDuk plugin. Trimmed reads were aligned to the SARS-CoV-2 reference genome MN908947. Variants present at frequencies of 1% or above were called using the Annotate and Predict Find Variations/SNPs in Geneious and verified by using the V-PIPE SARS-CoV-2 application (https://cbg-ethz.github.io/V-pipe/sars-cov-2/)(**28**).

Reads from MiSeq sequencing were processed as previously described⁶. Briefly, VSEARCH tools were used to merge paired reads and dereplicate sequences⁴⁴. Dereplicated sequences from RBD amplicons and rRNA templates were respectively mapped to the reference sequence of SARS-CoV-2 (NC_045512.2) Spike ORF or a collected reference index of animal mitochondrial and rRNA related sequences from NCBI's nucleotide and refseq databases (https://www.ncbi.nlm.nih.gov/) using either Bowtie2 or Minimap2. Mapped RBD amplicon sequences were then processed with SAM Refiner using the same Spike sequence as a reference and the command line

parameters '--alpha 1.8 --foldab 0.6'. The output from SAM Refiner were reviewed to determine the known and novel lineage makeup of the sampled sewersheds.

Mapped rRNA sequences were reviewed for matching of specific organisms. Sequences with poor mapping to sequences in the index and a random selection of sequences with good mapping were blasted to verify the organism match. Matches were corrected based on the blast results as needed.

Plasmids. Eukaryotic expression vectors for the heavy and light chains of antibodies LY-CoV016, LY-CoV555, and REGN10987 were obtained from Genscript. The lentiviral reporter constructed containing *Gaussia* luciferase (Gluc) with a reverse-intron (HIV-1-GLuc) was previously described⁴⁵. The codon optimized SARS-CoV-2 Spike expression vector was obtained from Tom Gallagher. This construct was modified to enhance transduction efficiency by truncating the last 19 amino acids, and introducing the D614G amino acid change. DNA gBlocks containing the WNY RBD sequences were synthesized by IDT and introduced into the SARS-CoV-2 expression construct using In-Fusion cloning (Takara Bio). Lentiviral Mouse and Rat Ace2 vectors pscALPSpuro-MmACE2 (Mouse) and pscALPSpuro-RnACE2 (Rat) were obtained from Jeremy Luban⁴⁶.

Cell culture. The 293FT cell line was obtained from Invitrogen. The 293FT+TMPRSS2 and 293FT+TMPRSS2+human Ace2 cells were previously described⁴⁷. All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 1 mM sodium pyruvate, 10 mM nonessential amino acids, and 1% minimal essential medium (MEM) vitamins. The ACE2 cell lines were generated by transfecting 293FT cells with 500 ng HIV GagPol expression vector, 400 ng of pscALPSpuro-MmACE2 (Mouse) or pscALPSpuro-RnACE2 (Rat), and 100 ng of VSV-G expression vector. Viral medium was used to transduce 293FT+TMPRSS2 cells⁴⁷, and cells were selected with puromycin (1 mg/mL) beginning 2 days postransduction and were maintained until control treated cells were all eliminated.

Monoclonal antibody synthesis. Transfections of 10cm dishes of 293FT cells were performed 5 mg each of heavy and light chain vectors and 40 mg polyethyleneimine (PEI)⁴⁸.

Virus production and infectivity assays. All transfections were performed in 10cm dishes. 293FT cells were transfected with a total of 9 mg of HIV-1-Gluc, 1 mg of CMV Spike vector, and 40 mg of PEI⁴⁸. Supernatants containing the virus were collected 2 days post-transfection. Transduction of ACE2 expressing cells was performed by plating 30,000 cells in 96 well plates and co-culturing with 50 mL of HIV-1-GLuc/Spike particles. Gluc was measured 2 days post-transduction.

Antibody Neutralization Assay. All blood collection and processing were performed under the approved protocols (MU Study of Serology for SARS-CoV-2 and MU COVID19 Vaccine study) by the Institutional Review Board of the University of Missouri. Written consent was received from all human subjects prior to being enrolled in the study. Subjects were requested to provide a date of positive PCR test for SARS-CoV-2 and subsequently had laboratory-based serologic tests to confirm the presence of antibody against SARS-CoV-2 S1 RBD protein. A total of 10-20 mL of blood was collected from each participant. The plasma was then separated from the blood cells by centrifugation and stored at -80°C.

Pseudovirus Neutralization Assay All human plasma samples were heat inactivated for 30 min at 56°C prior to the assay. Samples were diluted at 2-fold in 10 serial dilution in duplicates. Serially diluted samples were incubated with pre-titrated amounts of indicated pseudovirus at 37°C for 1 hour before addition of 293FT cells expressing human ACE2 and TMPRSS2 at 30,000 cells per well. Cells were incubated for 2 days and then the supernatant was used to measure gaussian luciferase (RLU). Infection was normalized to the wells infected with pseudovirus alone. Neutralization IC50 titers were calculated using nonlinear regression (Inhibitor vs normalized response—variable slope) in GraphPad Prism 9.0.

Data Availability

Raw sequencing reads are available in NCBI's Sequence Read Archive (SRA) under accession # PRJNA715712.

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Acknowledgments

The research described herein would not be possible if not for the assistance and support of a wide-range of organizations and individuals that came together to address the shared calamity that is the COVID-19 pandemic. We thank Jasmijn Baaijens, Michael Baym, Gina Behnke, Esmeraldo Castro, Francoise Chauvin, Alexander Clare, Pilar Domingo-Calap, Robert Corrigan, Pam Elardo, Raul Gonzalez, Crystal Hepp, Catherine Hoar, Dimitrios Katehis, William Kelly, Samantha McBride, Hope McGibbon, Hilary Millar, Jason Munshi-South, Samantha Patinella, Krish Ramalingam, Andrea Silverman, Jasmin Torres, Arvind Varsani, Peter Williamsen and members of the Dennehy Lab for for support, advice, discussions, and feedback. We also thank Molly Metz for assistance with graphics and figure design. This work was funded in part by the New York City Department of Environmental Protection and a donation from the Linda Markeloff Charitable Fund, and from the National Institutes of Health grant U01DA053893-01. The Water Research Foundation, the NSF Research Coordination Network for Wastewater Surveillance for SARS-CoV-2 and Qiagen Inc. provided resources, materials and supplies, technical support, and community support.

Author Information

Contributions

M.T., D.S.S., M.J., M.D. and J.J.D. supervised the project. M.T., D.S.S., M.J., and J.J.D. conceptualized the project. M.T., S.K., D.S.S., M.J., MD, and J.J.D. designed experiments. D.S.S., M.T., K.C., A.G., S.K., N.K., K.M.S., G.S., M.G., R.S., C.R., Y.G. and F.S. performed experiments. D.S.S., D.G., I.H., M.M., N.M., M.J., D.G. T.D.L. and J.J.D. performed data analysis and interpretation. M.T., D.S.S., MJ and J.J.D. wrote the original and revised manuscript drafts. All authors contributed to reviewing and editing of the manuscript.

Ethics Declarations

The authors declare no competing financial interests.

Additional Information

Supplementary Information is available for this paper.

Correspondence and requests for materials should be addressed to JJD or MJ.

Table 1. Predominant species detected in NYC wastewater via deep sequencing of 16S and 12S amplicons (nd = not detected).

Species	Common Name	WWTP 3	WWTP 10	WWTP 11	WWTP 12	
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Homo sapiens	Human	4/4	4/4	4/4	4/4
Bos taurus	Cow	4/4	2/4	3/4	4/4
Sus scrofa	Pig	3/4	3/4	4/4	1/4
Rattus norvegicus	Rat	3/4	nd	3/4	3/4
Canis familiaris	Dog	1/4	3/4	1/4	3/4
Gallus gallus	Chicken	2/4	2/4	nd	nd
Anas poecilorhyncha	Duck	nd	1/4	4/4	nd
Felis catus	Cat	1/4	1/4	2/4	nd
Ovis aries	Sheep	2/4	nd	nd	nd

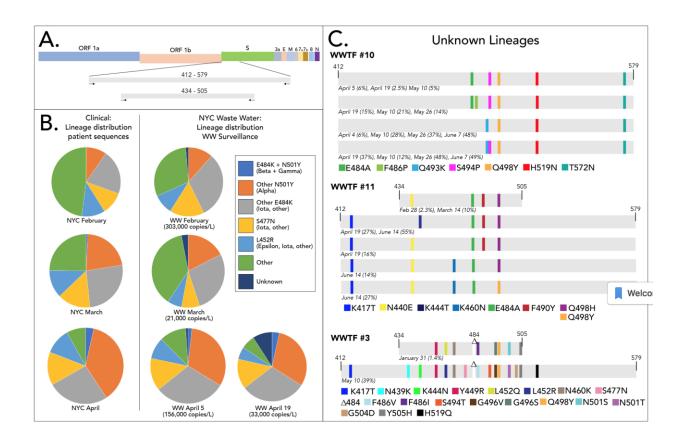


Figure 1. Novel SARS-CoV-2 lineages from Wastewater. A) Schematic of SARS-CoV-2 and the amplification locations. B) Distribution of SARS-COV-2 variants based on patient sequences and wastewater surveillance. C) Novel lineages detected. Schematic highlights shared sequences. Percentages indicate the percent of the sequences from each date that contained the indicated polymorphisms. Some sequences have irregular additional polymorphisms not listed.

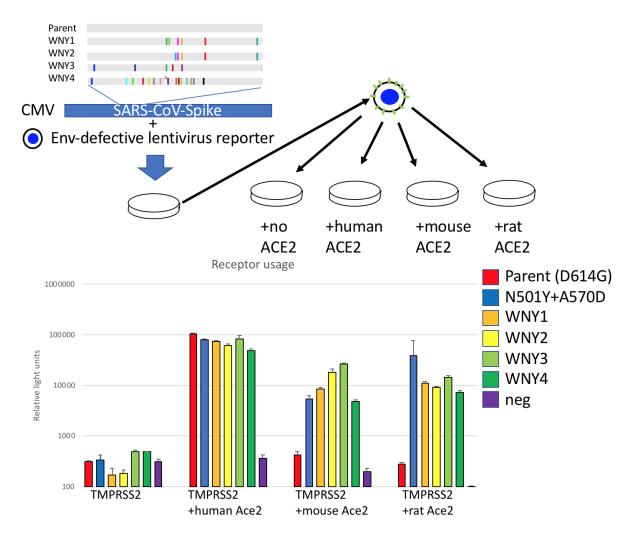


Figure 2. ACE2 usage by WNY lineages. A. Schematic of lineages and pseudovirus production. WNY1= E484A/ F486P/S494P/Q498Y/H519N/F572N, WNY2=Q493K/ S494P/Q498Y/H519N/T572N, WNY3= K417T/K444T/E484A/F590Y/Q498H, WNY4= K417T/N439K/K444N/Y449R/L452R/N460K/S477N/D484/F486V/S494T/G496V/Q498Y /N501T/G504D/505H/H519Q. Pseudovirus with indicated Spike proteins were generated and used to transduce 293FT+TMRPSS2 stably transduced with human, mouse or rat ACE2. Representative example of three experiments performed in triplicate.

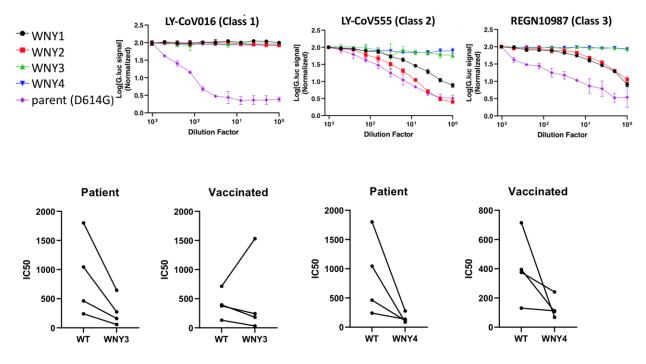


Figure 3. Antibody resistance to monoclonal neutralizing antibodies and patient serum. Lentiviral reporter pseudoviruses containing parent (D614F), WNY1, 2, 3, or 4 Spike proteins were treated with 2-fold dilutions of indicated monoclonal neutralizing antibody and used to infect 293FT+TMPRSS2+human ACE2. Representative example of 3 experiments performed in triplicate.

Supplementary Table 1. Mutations observed in NYC wastewater.

To be completed

Supplementary Table 2. Primers and probes used in this study.

Name and Site	Forward Primer (Probe)	Reverse Primer	Source
2019-nCoV_N1 (SARS-CoV-2 Spike)	GAC CCC AAA ATC AGC GAA AT	TCT GGT TAC TGC CAG TTG AAT CTG	
2019-nCoV_N1 Probe (SARS-CoV- 2 Spike)	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ		
RGlu2L/RCb9H (Rat Cytochrome B)	CAGCATTTAACTG TGACTAATGAC	TACACCTAGGAG GTCTTTAATTG	
EGL4L/RJ3R (Rat mtDNA D-loop)	CCACCATCAACA CCCAAAG	CATGCCTTGACG GCTATGTTG	
NTD sequencing primers (SARS- CoV-2 Spike n-terminal domain)	acactctttccctacacga cgctcttccgatctCATT CAACTCAGGACT TGTTCTT	gtgactggagttcagacg tgtgctcttccgatctCCA ATGGTTCTAAAGC CGAAA	
iSeq 100 RBD sequencing primers (SARS-CoV-2 Spike receptor binding domain)	TCGTCGGCAGCG TCAGATGTGTATA AGAGACAGccagat gattttacaggctgcg	GTCTCGTGGGCT CGGAGATGTGTA TAAGAGACAGgaa agtactactactctgtatg gttgg	
MiSeq RBD primary PCR primers (SARS-CoV-2 Spike receptor binding domain)	CTGCTTTACTAAT GTCTATGCAGATT C	TCCTGATAAAGAA CAGCAACCT	
MiSeq RBD Nested PCR primers (SARS-CoV-2 spike receptor binding domain)	acactctttccctacacga cgctcttccgatctGTGA TGAAGTCAGACA AATCGC	gtgactggagttcagacg tgtgctcttccgatctATG TCAAGAATCTCAA GTGTCTG	

12S-V5-Tailed-F1 and R1	TCGTCGGCAGCG TCAGATGTGTATA AGAGACAGACTG GGATTAGATACC CC	GTCTCGTGGGCT CGGAGATGTGTA TAAGAGACAGAG AACAGGCTCCTC TAG	
Taylor_16S_DEGE N_F1_Tailed Taylor_16S_DEGE N_R1_Tailed	TCGTCGGCAGCG TCAGATGTGTATA AGAGACAGGTTG GGGYGACYTYGG A	GTCTCGTGGGCT CGGAGATGTGTA TAAGAGACAGGC TGTTATCCCTRGR GTARC	
MiSeq 12s PCR primers	acactctttccctacacga cgctcttccgatctACTG GGATTAGATACC CC	gtgactggagttcagacg tgtgctcttccgatctTAG AACAGGCTCCTC TAG	
MiSeq 16s PCR primers	acactctttccctacacga cgctcttccgatctACC GTGCAAAGGTAG CATAAT	gtgactggagttcagacg tgtgctcttccgatctTCC GGTCTGAACTCA GATCAC	





Article Monitoring SARS-CoV-2 Populations in Wastewater by Amplicon Sequencing and Using the Novel Program SAM Refiner

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Abstract: Sequencing Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from wastewater has become a useful tool in monitoring the spread of viral variants. Approaches to this task have been varied, relying on differing sequencing methods and computational analyses. We used a novel computation workflow based on amplicon sequencing of SARS-CoV-2 spike domains in order to track viral populations in wastewater. As part of this workflow, we developed a program, SAM Refiner, that has a variety of outputs, including novel variant reporting as well as functions designed to remove polymerase chain reaction (PCR) generated chimeric sequences. With these methods, we were able to track viral population dynamics over time. We report here on the emergence of two variants of concern, B.1.1.7 (Alpha) and P.1 (Gamma), and their displacement of the D614G B.1 variant in a Missouri sewershed.

Keywords: coronavirus; wastewater; metagenomics; molecular epidemiology

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused a pandemic and a worldwide health crisis starting in 2020 [1]. Full genome sequences of SARS-CoV-2 were rapidly made available within the first months of spread [2,3]. Partial- and wholegenome sequencing of SARS-CoV-2 have been important tools in monitoring transmission paths and the emergence of variant lineages. Sequencing of SARS-CoV-2 has mostly been performed using clinical samples. However, early in the SARS-CoV-2 pandemic, wastewater was used to track community levels and spread of SARS-CoV-2 by Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) methods [4,5]. Investigators have also used high-throughput sequencing on wastewater samples to obtain full or partial SARS-CoV-2 genomic sequences which have been used for metagenomic and epidemiologic analysis [6–13]. Sequences identified in wastewater samples may reflect known lineages as well as lineages not reported from clinical samples. Combinations of mutations not observed in clinical samples may represent new infections not yet picked up by clinical sampling or lineages that are under-represented in clinical samples. Approaches using wastewater are particularly relevant with the emergence of variant lineages that may vary from previous isolates in their fitness and/or pathogenesis.

The state of Missouri has been monitoring wastewater to track the prevalence and spread of SARS-CoV-2 using RT-qPCR (https://storymaps.arcgis.com/stories/f7f54924 86114da6b5d6fdc07f81aacf accessed on 23 June 2021). We sought to begin using the same



Citation: Gregory, D.A.; Wieberg, C.G.; Wenzel, J.; Lin, C.-H.; Johnson, M.C. Monitoring SARS-CoV-2 Populations in Wastewater by Amplicon Sequencing and Using the Novel Program SAM Refiner. *Viruses* 2021, *13*, 1647. https://doi.org/ 10.3390/v13081647

Academic Editor: Oliver Schildgen

Received: 23 June 2021 Accepted: 17 August 2021 Published: 19 August 2021

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). samples for high-throughput sequencing to track the presence and spread of known and previously unreported variant lineages. We were specifically interested in the spike gene, so we used primers to target 3 regions for amplification, the N-terminal domain (NTD), the receptor binding domain (RBD) and the region of the S1 and S2 subunit split (S1S2). We chose these regions due to the numerous variations matching evolving lineages found in them and their significance in potential immune evasion [14]. While there are a number of high-throughput sequencing technologies and methods, the sequencing output is relatively standard, whereas the processing and analysis of that sequence data are not. There are numerous programs and pipelines that can be used to obtain information from sequences and remove errors generated from PCR, such as single-nucleotide (nt) polymorphisms (SNPs) and chimeric sequences. While many of these are quality approaches, we were unable to find a simple program or workflow with existing programs for high-throughput sequencing data that produced a condensed report of known and unknown co-variants found in the data. We wanted the variant report to include SNPs, multiple nucleotide polymorphisms (MNPs), insertion and deletion events (indels), and downstream amino acid changes, and also wanted PCR-generated chimeric sequences removed. While some programs or pipelines partially fulfilled these criteria, none we found did so fully.

Here, we detail the workflow we used to analyze high-throughput sequencing data and the program we developed to provide a human-readable, information-dense output for viewing lineages that meet the criteria described above. Using this workflow and our program, we were able to monitor SARS-CoV-2 population changes in a Missouri sewershed.

2. Materials and Methods

2.1. Wastewater Collection

Twenty-four-hour composite samples were collected at wastewater treatment facilities (WWTF) and maintained at 4 °C until they were delivered to the analysis lab, generally within 24 h of collection. Samples reported in this study were collected at the NPSD Interim Saline Creek Regional WWTF in Fenton, MO, USA.

2.2. RNA Extraction

Wastewater samples were centrifuged at $3000 \times g$ for 10 min and then filtered through a 0.22 µM polyethersolfone membrane (Millipore, Burlington, MA, USA). Approximately 37.5 mL of wastewater was mixed with 12.5 mL solution containing 50% (w/vol) polyethylene glycol 8000 and 1.2 M NaCl, mixed, and incubated at 4 °C for at least 1 h. Samples were then centrifuged at 12,000 × g for 2 h at 4 °C. Supernatant was decanted and RNA was extracted from the remaining pellet (usually not visible) with the QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD, USA) using the manufacturer's instructions. RNA was extracted in a final volume of 60 µL.

2.3. Sequencing

The primary RT-PCR (25 μ L) was performed with 5 μ L of RNA extracted from wastewater samples with loci-specific primers (0.5 μ M each) (Table 1) using the Superscript IV One-Step RT-PCR System (Thermo Fisher, Waltham, MA, USA). Primary RT-PCR amplification was performed as follows: 25 °C(2:00) + 50 °C(20:00) + 95 °C(2:00) + [95 °C(0:15) + 55 °C(0:30) + 72 °C(1:00)] × 25 cycles. Secondary PCR (25 μ L) was performed using 5 uL of the primary PCR as template with gene-specific primers containing 5' adapter sequences (0.5 μ M each), dNTPs (100 μ M each) and Q5 DNA polymerase (NEB, Ipswich, MA, USA). Secondary PCR amplification was performed as follows: 95 °C(2:00) + [95 °C(0:15) + 55 °C(0:30) + 72 °C(1:00)] × 20 cycles. A tertiary PCR (50 μ L) was performed to add adapter sequences required for Illumina cluster generation with forward and reverse primers (0.2 μ M each), dNTPs (200 μ M each), and Phusion High-Fidelity DNA Polymerase (1U). PCR amplification was performed as follows: 98 °C(3:00) + [98 °C(0:15) + 50 °C(0:30) + 72 °C(0:30)] × 7 cycles + 72 °C(7:00). The amplified product (10 μ L) from each PCR reaction is combined and thoroughly mixed to make a single pool. Pooled amplicons were purified by addition of Axygen AxyPrep MagPCR Clean-up beads in a 1.0 ratio to purify final amplicons. The final amplicon library pool was evaluated using the Agilent Fragment Analyzer automated electrophoresis system, quantified using the Qubit HS dsDNA assay (Invitrogen, Waltham, MA, USA), and diluted according to Illumina's standard protocol. An Illumina MiSeq instrument was used to generate paired-end 300 base pair length reads. Adapter sequences were trimmed from output sequences using cutadapt [15]. The raw and trimmed reads for the samples used in this report are available at https://github.com/degregory/SR_manuscript/tree/master/Fenton_Data accessed on 23 June 2021. Raw reads for all of Missouri wastewater monitoring will be available under BioProject PRJNA748354.

Table 1. PCR primers used to amplify spike regions for MiSeq sequencing. Upper-case lettering indicates SARS-CoV-2 sequence. Lower-case lettering indicates adapter sequence.

Region	PCR	Orienation	Primer Sequences
RBD	Primary	forward	CTGCTTTACTAATGTCTATGCAGATTC
	Primary	reverse	TCCTGATAAAGAACAGCAACCT
	Secondary	forward	acactctttccctacacgacgctcttccgatctGTGATGAAGTCAGACAAATCGC
	Secondary	reverse	gtgactggagttcagacgtgtgctcttccgatctATGTCAAGAATCTCAAGTGTCTG
NTD	Primary	forward	GTGGTGTTTATTACCCTGACAAAG
	Primary	reverse	GCTGTCCAACCTGAAGAAGA
	Secondary	forward	acactctttccctacacgacgctcttccgatctCATTCAACTCAGGACTTGTTCTT
	Secondary	reverse	gtgactggagttcagacgtgtgctcttccgatctCCAATGGTTCTAAAGCCGAAA
S1S2	Primary	forward	GCCGGTAGCACACCTTGTAA
	Primary	reverse	TGTGCAAAAACTTCTTGGGTGT
	Secondary	forward	cactetttecetacaegaegetetteegatetCAGGCACAGGTGTTCTTACT
	Secondary	reverse	gtgactggagttcagacgtgtgctcttccgatctGTCTTGGTCATAGACACTGGTAG

3. Results

3.1. Computational Pre-Processing

Figure 1 illustrates the steps of our workflow. The two steps following read trimming used the VSEARCH tool [16]. First, the trimmed paired reads were merged using vsearch 'fastq_merge' with default parameters. Merged reads were then dereplicated using vsearch '-derep_fullength' with the arguments '-minsize 100' and '-sizeout'. These arguments limit the output to unique sequences that occur at least 100 times and appends the sequence IDs with 'size=#', where # is the number of times that particular sequence occurred in the reads. The cutoff of 100 counts removes late-stage PCR errors, leaving only sequences representing the original templates or errors that occurred in early cycles of the PCR. This removal makes further analysis simpler and faster. However, very low frequency original template sequences will also be removed by this cutoff, so this step could be skipped to preserve such rare sequences. The resulting unique sequences were mapped to the sequence of SARS-CoV-2 (NCBI Reference Sequence: NC_045512.2, https://www.ncbi.nlm.nih.gov/nuccore/ NC_045512, accessed on 7 February 2021) spike ORF using Bowtie2 [17] with default parameters to generate standard SAM formatted files. Having SAM formatted files allows the use of the program we developed for amplicon sequencing results. All files associated with these steps for our analysis of the Fenton, MO sewershed in this manuscript can be accessed at https://github.com/degregory/SR_manuscript/tree/master/Fenton_Data accessed on 23 June 2021.

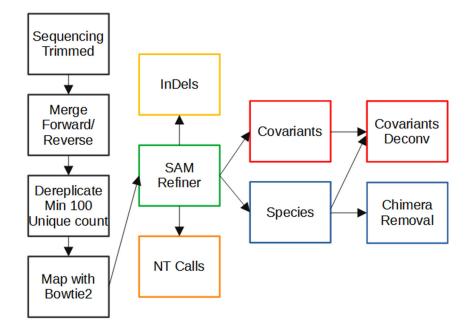


Figure 1. Workflow of Amplicon Sequencing Analysis. Computational processing of sequencing results prior to the use of SAM Refiner is seen in the black boxes. Paired end reads generated from an Illumina MiSeq were trimmed of low-quality calls at the end of the reads. Paired end reads were then merged into single sequence reads. Reads were then dereplicated to unique sequences with at least 100 counts while preserving the count information in the sequence IDs. Dereplicated sequences were then mapped to the sequence of the SARS-CoV-2 spike ORF using Bowtie2. SAM Refiner was then used to process the mapped reads to obtain information about the variant lineages observed, initially outputting 4 TSV files to report unique sequences, nt calls, indels and covariants. The unique sequences and covariants were further processed to remove chimeric PCR artifacts to produce covariant deconvolution and chimera removed outputs.

3.2. SAM Refiner: SAM Processing

Our program, SAM Refiner, is currently a command line-based python script and is available at https://github.com/degregory/SAM_Refiner accessed on 23 June 2021 along with updated documentation. In order to run SAM Refiner, a python compiler or interpreter is needed (https://docs.python.org/3/tutorial/interpreter.html accessed on 23 June 2021). Though only tested in a Linux environment, it should function with other common operating systems. Figure 2 shows the command line usage for SAM Refiner. Standard SAM formatted files are the starting point for our program. These files are generated by many mapping programs, including Bowtie2 [17] and BWA [18]. The default functions of SAM Refiner follow. Files with the extension .sam (case insensitive) in the working directory will be identified and processed. To process SAM files, SAM Refiner must be provided a FASTA formatted file for a reference sequence using the command line argument '-r reference.fasta', where the FASTA file contains the same sequence ID and sequence used to map the sequencing reads in the SAM formatted file. If the IDs of the given reference and the reference of mapped sequences in the SAM file do not match, those sequences will be ignored. If the SAM formatted files were generated from dereplicated or collapsed sequences that contain the unique read count in sequence ids where the count is at the end of the id and denoted with a '=' or '-', SAM Refiner will recognize the counts, i.e., 'Seq1:1; counts = 20' will be recognized as a sequence with 20 occurrences.

```
Spython SAM. Refiner py -h
usage: SAM_Refiner py [h] [-REF] [S [SAM_FILES [SM_FILES ...]] [-use_count (0.1)]
[-mm_abudnated iNM_ABUNDANCE1] [-min_abundance2 MIN_ABUNDANCE2] [-ntabund NTABUND]
[-mm_abudnated iNM_ABUNDANCE1] [-min_accovar MAX_COVAR] [-Arapopt (0.1)] [-Acadonas MNP [0.1)]
[-max.dist MAX_DIST] [-max_covar MAX_COVAR] [-Arapopt (0.1)] [-Acadonas MNP [0.1)]
[-max.dist MAX_DIST] [-max_covar MAX_COVAR] [-Atappot (0.1)] [-Acadonas MNP [0.1)]
[-max.dist MAX_DIST] [-max_covar MAX_COVAR] [-Atappot (0.1)] [-Acadonas MNP [0.1)]
[-max_covar MAX_COVES] [-bata BETA] [-autopass AUTOPASS] [-coliD COLID] [-collect (0.1)]
[-max_covar MIN_BIND[ -lipha ALPMA] [-list(0.1)] [-li
```

Figure 2. Command Line Usage of SAM Refiner. The standard help output from SAM Refiner is shown. Syntax for the command line usage is seen followed by details about potential arguments to modify program parameters.

For each SAM file, SAM Refiner initially outputs 4 tab separated value (TSV) files that can be read by any standard spreadsheet software. For a SAM file with the name Sample.sam, the outputs are named Sample_unique_seqs.tsv, Sample_nt_calls.tsv, Sample_indels.tsv and Sample_covars.tsv. Example outputs of each are provided in Supplementary Files 1, 2, 3, and 4, respectively (https://github.com/degregory/SR_manuscript/tree/master/Supplementals accessed on 23 June 2021). All reports are based on the FASTA reference relative to the SAM formatted file, so any errors made by the mapping or incongruence between the FASTA reference and the mapping reference will result in propagated errors. The reports also include the coded amino acids and their position in the coded peptide as if the reference is an in-frame coding sequence. If multiple nucleotides in a single codon differ from the reference, they will be reported together as a MNP with the associated amino acid change. Within the files, all of the sample-specific outputs start with the name of the sample taken from the SAM file name followed in parenthesis by the count of reads mapped.

The Sample_unique_seqs.tsv file (Supplementary 1) lists the unique sequence reads mapped in the SAM file using a variant notation to list the variations from the reference along with occurrence count and abundance. For example, using the previously mentioned SARS-CoV-2 spike ORF as the reference sequence, a sequence read that matches the reference except for having a T at position 1501 instead of the reference A would be reported simply as '1501A(N501Y)'. The abundance reported uses decimal notation, so 0.2 represents 20% abundance. Unique sequences that have an abundance below 0.001 are not reported.

The Sample_nt_calls.tsv file (Supplementary 2) has a line for each nt position covered in at least 0.1% of the reads. Based on the reference sequence, each line first reports the nt position, the reference nt, the encoded amino acid position, and the amino acid residue encoded by the reference sequence. The line then reports the number of calls for each base and for deletions at that position, followed by the most abundant (primary) call and its counts and abundance. If the primary nt is different from the reference sequence, the amino acids encoded by the primary nt sequence and by the reference sequence with only that nt changed are reported. Further, if the second (secondary) and third (tertiary) most abundant nts are above 0.1% of the total read counts, those nts, their counts, abundances, and associated amino acid changes are also reported.

The Sample_indels.tsv (Supplementary 3) file lists each insertion or deletion found in the mapping along with its occurrence count and abundance. Reported insertions have the format of 'position-insertNT(s)', so an insertion between nt positions 54 and 55 of the sequence 'GCA' will be reported as '55-insertGCA'. Reported deletions have the format 'start Position-end positionDel', so a deletion of the nts at positions 61 through 64 would be reported as '61-64Del'. Amino acid changes are reported if the indel maintains the reading frame. If there are no indels in the reads, no indel report will be generated.

Finally, the Sample_covars.tsv (Supplementary 4) file lists all observed single polymorphisms and polymorphisms combinations relative to the reference sequence. The number and abundance of sequence reads containing each covariant (covar) are reported regardless of whether any of those reads have other variations or not. As an example of this processing, the sequence '1212G(G404G) 1501T(N501Y) 1709A(A570D)' with 100 counts would have the covariants of '1212G(G404G)', '1501T(N501Y)', '1709A(A570D)', '1212G(G404G) 1501T(N501Y)', '1709A(A570D)', '1212G(G404G) 1501T(N501Y)', '1209A(A570D)', '1212G(G404G) 1501T(N501Y)', '1209A(A570D)', and '1212G(G404G) 1501T(N501Y) 1709A(A570D)', and contribute 100 counts to each. Because unique sequences that fall below the 0.1% reporting cutoff can still contribute to covariants, there may be polymorphisms in the reported covariants that are not seen in the unique sequence output. Any sequences with more than 40 polymorphisms are analyzed, only combinations of 8 or fewer polymorphisms are reported.

Once the above outputs are generated from each SAM file found, SAM Refiner will collect information from each sample and report them in a single file for the covars and unique_seqs reports (Collected_Covariances.tsv and Collected_Unique_Seqs.tsv). These collections have a threshold of 1% occurrence for reporting.

Many options are available as command line arguments that can change parameters of SAM processing of SAM Refiner (Figure 2). There are no strictly required command line arguments, though the '-r' argument is required for the SAM processing. Omitting the reference sequence will cause SAM Refiner to skip SAM processing and only perform the collections and chimera removal (see below), which require pre-existing outputs. The other input option is the '-S' argument, which provides SAM Refiner with SAM files to process instead of searching the working directory. The use of dereplicated/collapsed counts in the SAM files can be disabled by using '-use_counts 0'. There are also options available for the outputs. All outputs can be separately suppressed with the arguments '-seq 0', '-nt_call 0', '-indel 0', '-covar 0' and '-collect 0'. The collections file names can be prepended with a string specified by the argument '-colID'. To change the reporting threshold for the sample and collected outputs, arguments '-min_abundance1' and '-min_abundance2' are used, respectively. For '-min_abundance1', despite its name, the value can be used to either set a minimal abundance threshold or a minimal count threshold. Values of 1 or greater will set a count threshold, while those less than 1 will set an abundance threshold. Only an abundance threshold is available for '-min_abundance2'. All amino acid information in the reports can be suppressed with the argument '-AAreport 0', which is recommended if the reference does not primarily provide an in-frame coding sequence. Users can also have all nt changes processed independently, even if they are in the same codon, with '-AAcodonasMNP 0'. Using '-ntabund' will change the required mapped coverage threshold for reporting a position in the nt_calls output. Finally, '-max_dist' and '-max_covar' allow changes to covar processing and reporting. Sequences with more variations than the amount specified by '-max_dist' are not included in the covar analysis. The maximum number of polymorphisms reported in a combination can be set with '-max_covar'. As an example, if '-max_covar 2' were used for Sup. 4, then '1216-1216Del 1501T(N501Y) 1709A(A570D)', '1212G(G404G) 1501T(N501Y) 1709A(A570D)' and '1217-1217Del 1501T(N501Y) 1709A(A570D)' would not be reported.

Using the SAM files generated from the sequencing data of the Fenton sewershed, we ran SAM Refiner with the same reference as was used for Bowtie2 mapping, the SARS-CoV-2 (NCBI Reference Sequence: NC_045512.2) spike ORF sequence. The resulting outputs can be accessed at https://github.com/degregory/SR_manuscript/tree/master/Fenton_Data accessed on 23 June 2021. These outputs allow us to see the variant lineages present at different dates in this sewer shed. However, as can be seen in Supplementary 1, many of the sequences reported appear to be chimeric sequences arising from template jumping. While these outputs can still be used for further analysis, removing chimeric sequences makes such analysis easier, so SAM Refiner also has methods to remove such chimeric sequences.

3.3. SAM Refiner: Chimera Removal

PCR amplification can introduce sequence errors that obscure the original template sequences. Of most concern are the introduction of false SNPs and chimeric reads. Most PCR-introduced SNPs can be removed from analysis by the use of an abundance threshold such as is the default for SAM Refiner, or as was used in our pre-processing dereplication step. There are also numerous other programs that can be used to attempt to remove such errors. Chimeric sequences are generally more difficult to remove. Many programs exist for this task; however, we were unable to find any that provided satisfying results for our amplicon sequencing. We developed two algorithms for SAM Refiner in order to remove chimeric errors arising from PCR template jumping from the SAM processing outputs. They are redundant in their function but crosschecking between the two different methods allows for increased confidence in the results.

The algorithms to remove chimeric sequences rely on the unique sequence and covariant files generated by SAM processing. The first algorithm, chimera removed (chim rm), goes through the individual unique sequences, starting with the lowest abundance, to determine if the sequences are chimeric. Figure 3 shows a simplified example of how the determination is made on the lowest abundant sequence of an example unique sequence output (Supplementary 5). For this step, the sequence being considered as a potential chimera is broken up into all possible dimeric halves. Each pair is then compared to all the other sequences to detect potential parents. A sequence is flagged as a potential parent if its abundance is greater than or equal to the abundance of the potential chimera multiplied by 1.8 (foldab) and there is at least one other sequence that would be a matched parent to the complimentary dimeric half. When a pair of dimeric halves have potential parents, the abundances of parent pairs are multiplied. The products from each potential parent pairings are summed as an expected abundance value and compared to the observed abundance of the potential chimera. If the abundance of the potential chimera is less than that of the expected value multiplied by 1.2 (alpha), that sequence is flagged as a chimera and removed. The counts attributed to the flagged chimeric sequence are then redistributed to the parent sequences based on the relative expected contribution to recombination. Once this process has been performed for all the sequences, it is repeated until no more sequences are flagged as chimeric or 100 chimera removal cycles have completed. The results of this algorithm that have a recalculated abundance of 0.001 or greater are output in a new file (Supplementary 6 Example_a1.2f1.8rd1_chim_rm.tsv). The added string represents values of the parameters used for the processing (alpha, foldab and redist; see below for more information on the parameters).

Variant Seque 1450A(E484K) 1709 Potentia		Counts 1478 arent Pairs	Abundance 0.006			
Left Parent : Abundance 1450A(E484K) : 0.07		parent : Abunc) / 1709A(A570			Multiplied Abundance 0.03402 (73%)	
1450A(E484K) : 0.07	1709A(A570	D): 0.097			0.00679 (14%)	
1450A(E484K) : 0.07	1450A(E484K) 1501T(N501Y) / 1709A(A570D) : 0.0	033	0.00231 (5%)	
1450A(E484K) / 1501T(N501Y) 1709A(A570D) : 0.033	1709A(A570	D): 0.097			0.003201 (7%)	
1450A(E484K) / 1501T(N501Y) : 0.006	1709A(A570	D): 0.097			0.000582 (1%)	
Query (actual) Abundance 0.006 1450A(E484K) 1709A(A570I	< .	.046903		Total:	: 0.046903 (100%)

Figure 3. First Method of Detection and Removal of Chimeras, Chimera Removed. Using the sequences shown in Supplementary 5, the query of the least abundant sequence is shown. Potential parents whose recombination could result in the query sequence are found. The abundances of each potential pair are multiplied. The sum of the multiplied abundances of the pairs (expected) is then compared to the abundance of the query sequence (actual) to determine if the query sequence is a chimera. If the actual abundance is greater or equal to 1.2-fold the expected abundance, the sequence is considered non-chimeric.

The second algorithm, covariant deconvolution (covar deconv), is a two-step process. Figure 4 shows these processes using the example outputs found in Supplementary 5 and 7. The first step determines if a sequence is likely to be a true or chimeric sequence by obtaining the ratio of the frequency of a given covariant sequence relative to an expected abundance of that covariant sequence assuming random recombination of its individual polymorphisms. The expected abundance is obtained by multiplying the abundances of each individual polymorphism that is present in that covariant sequence. For instance, in a sample where '1501T(N501Y)' has an abundance of 0.32 and '1709A(A570D)' has an abundance of 0.35, the expected abundance of the covariant '1501T(N501Y) 1709A(A570D)' would be 0.112 $[0.32 \times 0.35]$. If the ratio of the observed abundance to the expected abundance is equal to or greater than 1 (beta), that covariant passes the check and is sent to the second step. Any sequence that has an abundance of 0.3 or greater is automatically passed. If such a sequence has an observed/expected ratio less than 1, it will be assigned a ratio of 1. The second step processes the passed sequences in order of greatest observed/expected ratio to least. If multiple sequences have the same ratio, they are processed in order of greatest to least distance from the reference. Sequences that automatically pass the first step are processed after the other sequences in order of least abundant to greatest. Sequences are assigned a new occurrence count based on their constituent individual polymorphisms. For the sequence being processed, the count for the least abundant individual polymorphism is assigned to the sequence and constituent polymorphisms making up the sequence have their count reduced by the amount of the least abundant polymorphism. This reduction means the individual polymorphism that had the least counts is assigned 0 counts, so any sequence not yet processed in which that polymorphism is present is functionally removed. This process is repeated until all sequences have been reassessed or removed. The final results with an abundance of 0.001 or greater are reported in a new file (Supplementary 8 Example_covar_deconv.tsv).

A 1450Ą(E48	4K) 1501	Γ <mark>(N</mark> 501Y) 17	09A(A570D)	
Sequence abundance				0.033 O	bserved
Singles abundance 0.115	× 0.	626 ×	0.621	= 0.037 Ex	kpected
			Ratio:	0.033 / 0.0	37 = 0.890
B Unique Sequence		Abundanc	e Singles	Ratio	Pass/Fail
1450A(E484K)		0.115	N/A	1	Pass
1450A(E484K) 1501T(N501Y)		0.039	0.059	0.653	Fail
1450A(E484K) 1501T(N501Y) 1709	A(A570D)	0.033	0.037	0.890	Fail
1450A(E484K) 1709A(A570D)		0.038	0.071	0.532	Fail
1501T(N501Y)		0.626	N/A	1	Pass
1501T(N501Y) 1709A(A570D)		0.519	0.322	1.610	Pass
1709A(A570D)		0.621	N/A	1	Pass
Reference		0.202	N/A	1	Pass
C Sequence	Count				
1501T(N501Y) 1709A(A570D)	136755				
1501T(N501Y)	165000				
1709A(A570D)	163714				
1501T(N501Y) 1709A(A570D)	163714				
1501T(N501Y) 165000 - 163714	1286				
1709A(A570D)	0				

Figure 4. Second Chimera Removal Method in SAM Refiner, Covariant Deconvolution. (**A**) Calculations of the singles/expected abundance and abundance ratio for one of the unique sequences from Sup. 5 and the abundances from Sup. 7. Lines connect the singles and their abundance to the same in (**B**). (**B**) Calculations for determining if a unique sequence passes the initial check. Sequences pass when they have an abundance/singles ratio of 1 or greater. (**C**) Passed sequences are processed in order of greatest ratio to least. Counts of the sequence are set to the counts of the least abundant single variant, and that count is then removed from all single variants in that sequence.

As before, the results from individual samples are collected and reported for entries above 1% occurrence. A number of command line arguments will also influence the chimera removal algorithms. Both chimera removal algorithms run by default, but either or both steps can be disabled ('-chim_rm 0' and '-covar_deconv 0'). The collections are again disabled with '-collect 0'. An additional output of the covariants that passed the first step of the second algorithm can be generated with '-pass_out 1' (Supplementary 9). The outputs are constrained as before by a minimum abundance with command line arguments '-min_abundance1' and '-min_abundance2'. Collection file names are also prepended with '-colID'. The only input parameter that can be changed by command line argument is the abundance of sequences or covariants that will be considered in the algorithms. By default, only entries from the inputs that have a 0.001 abundance or greater are processed. This threshold can be changed with '-chim_in_abunda'.

Four parameters can be altered for the first algorithm. The abundance ratio that is used as a threshold for selecting potential parents of a potential chimera can be set with '-foldab'. Larger values will generally reduce the pool of sequences that will be considered as potential parents, thus potentially reducing the total expected abundance obtained from parent pairs and the number of sequences flagged as chimeric. In the simplest theoretical model of PCR chimera generation, two parents generate one chimera. The parents have at least twice the abundance of the chimera as they would exist and have been amplified prior to the chimera, but the reality of chimera generation can be much more complex as many sequences may generate identical chimeras multiple times. If a sample has little chimera generation, a '-foldab' value close to 2, such as the default of 1.8, should be sufficient to remove chimera generation observed, the more the '-foldab' value needs to be reduced to accurately remove all chimeric sequences. Though it would be rare, this value can even be set to 0 so as not to exclude any sequence from being considered a potential parent. Lower

values, however, will also increase the likelihood of a sequence being flagged as a chimera in error. Users may need to empirically determine the best value for their samples.

The multiplier for the parental summed abundance for determining if a sequence is a chimera can be set with '–alpha'. Larger values will generally result in a greater number of sequences flagged as chimeric. As with '–foldab', the optimal value for '–alpha' will depend on the extent of chimera generation in the samples being processed, with a value near 1 for minimal chimera generation (such as the default 1.2) and 2 or even higher for rampant chimera generation. Once again, the later would also increase the likelihood of sequences being flagged as chimeric in error.

Redistribution of the counts from the chimera to the parent sequences can be disabled with '-redist 0'. Redistribution is meant to give an estimate of the counts and abundances that would have been observed without chimera generation which users may wish to forgo. The maximum number of chimera removal cycles can be changed by '-max_cycles', (i.e., '-max_cycles 2' will only allow two iterations of the chimera removal). Multiple removal cycles allow chimeras to be found based on new counts and abundances resulting from previous cycles, increasing the likelihood chimeras are removed from a sample.

The second algorithm has two parameters that can be changed. The ratio threshold at which a covariant will be passed to the second step can be altered with '-beta'. The abundance at which a covariant will automatically be passed can be changed with '-autopass'.

The chimera removal methods of SAM Refiner were also used on the Fenton sewershed sequencing data. Due to the relatively high amount of chimeric sequences in our samples, we used the command line arguments '-foldab = 0.6 -alpha = 2.2'. The outputs generated for the Fenton sewershed from 2-2-21 to 4-13-21 can be accessed at https://github.com/degregory/SR_manuscript/tree/master/Fenton_Data accessed on 23 June 2021. The two different chimera removal methods showed good concordance, validating each as being a viable detection method. Duplicate RT-PCR preparation and sequencing of the same wastewater sample also generally provided similar results, though less consistently (Figure 5. Compare A and B RBD amplicon preparations). These differences were more pronounced with covariants with relatively low abundance, such as is seen with 3-30 RBD samples, where one detects T478K and the other does not (Figure 5). These differences illustrate the stochastic nature of RT-PCR amplification.

We used the chimera removed and covariant deconvolution outputs to assign sequences to known variant lineages or the reference (Supplementary 10, 11 and 12) based on polymorphisms present. Polymorphisms were considered for lineage assignment if they appeared in multiple sequencing runs or were known to be present in circulating populations reported to GSIAD (https://www.gisaid.org/, accessed on 20 February 2021). Polymorphisms that could not be validated were not taken into account for lineage assignment. Based on these assignments, we were able to observe the changes to virus populations in the sewershed over time (Figure 5). We classified the sequences found from the NTD amplicon as matching reference sequence, lineage B.1.1.7 (Alpha) with '203-208Del 429-431Del' or lineage P.1 (Gamma) with '412T(D138Y) 570T(R190S)' (Supplementary 10). Sequences from the RBD amplicon matched reference sequence, lineages B.1.1.7 with '1501T(N501Y) 1709A(A570D)', P.1 with '1250C(K417T) 1450A(E484K) 1501T(N501Y)', or had the single variations of T478K or L452R (Supplementary 11). T478K and L452R each have lineage associations. However, no other polymorphisms are associated with these in the RBD amplicons, nor were any polymorphisms present in the other amplicons that would indicate the presence of any associated lineages. While these SNPs could be the result of PCR error, it is more likely the associated lineages exist in the sewershed, but, due to stochastic effects, the other associated polymorphisms in the other amplicons were not detected. They could have also arisen in a reference background. As we cannot assign them to a known lineage with any certainty, we assigned them to their own category. Sequences from the S1S2 amplicon matched lineage B.1.1.7 with '1841G(D614G) 2042A(P681H) 2147T(T716I)', lineage P.1 with '1841G(D614G) 1963T(H655Y) 2063T(A688V)' or the B.1 lineage with only the now ubiquitous D614G variation (Supplementary 12). The 03-23 S1S2 sample had a sequence '1841G(D614G) 2037G(N679K) 2063T(A688V)'. While A688V is associated with P.1, it does not appear in that context here. As that is the only sample where those covariant sequences were observed and the polymorphisms are not frequently reported in GISAID (outside of P.1 for A688V), we did not feel we could validate this sequence as a novel lineage and instead tentatively assigned it to the reference category. From these results, we can conclude that the SARS-CoV-2 population of this sewershed changed in March 2021 from almost exclusively the D614G B.1 lineage to mainly the B.1.1.7 lineage, with the introduction of P.1 early in April 2021. This general method is now being used to track SARS-CoV-2 variants in many Missouri sewersheds (https://storymaps.arcgis.com/stories/f7f5492486114da6b5d6fdc07f81aacf accessed on 23 June 2021).

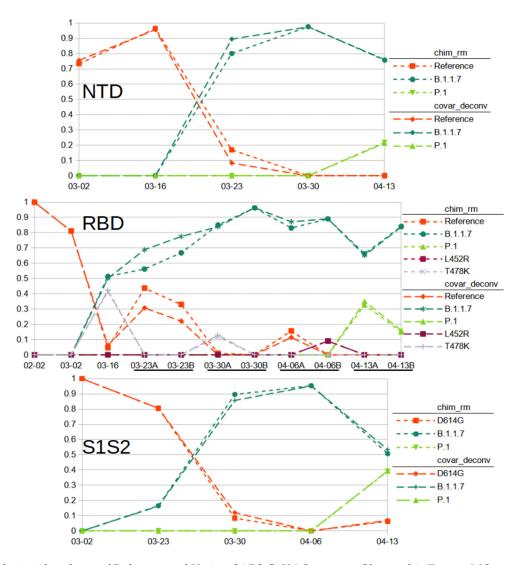


Figure 5. Relative Abundance of Reference and Variant SARS-CoV-2 Sequences Observed in Fenton, MO sewershed from February to March. Results from sequencing of spike amplicons of the NTD, RBD and S1S2 junction regions are shown. Lines of short dashes connect values obtained by the chimera removed method, lines of long dashes connect values obtained by the covariant deconvolution method. All amplicons show a population shift from the reference with D614G to B.1.1.7 sequences with the appearance of P.1 sequences at the last time point. Additionally, known common polymorphisms T478K and L452R were observed from the RBD amplicons. RT-PCR for the RBD amplicon was performed in duplicate for some samples.

4. Discussion

4.1. General Discussion

Especially as new SARS-CoV-2 variants emerge that have altered viral fitness and/or pathogenesis, it is important for health professionals and policy makers to have up-to-date information on the viral populations present in communities. Surveillance of wastewater by high-throughput sequencing has proven to be a cost effective and reliable method to obtain such information [6–13]. Sequencing of wastewater for SARS-CoV-2 relies on whole-genome sequencing, targeted amplicon sequencing, or some intermediate of the two; each approach has advantages and disadvantages. Whole-genome sequencing is more likely to detect polymorphisms across the whole genome that are present in a local viral population. However, the ability to link individual polymorphisms to each other is negatively impacted by distance. The difficulty in linking polymorphisms can hinder identifying specific lineages in a population. Targeted amplicon sequencing only provides information on the targeted sequence. However, polymorphisms within the target can be easily linked and lead to easier lineage identification if the targeted sequence(s) are rich in lineage-defining polymorphisms. The spike gene, particularly the regions encoding the NTD, RBD, and S1S2 junction, is rich in such polymorphisms.

We choose these regions for targeted amplicon sequencing in order to identify lineages present in Missouri communities. This approach has proven effective in combination with our computation workflow, and we have reported here our finding for one Fenton, MO sewershed. Our results readily demonstrate the changes in this community's viral population over time. Based on the ability to readily detect variants, our methods should also detect novel variants that have polymorphisms in these regions.

Beyond this specific application, our methods may be generalized to monitoring wastewater for variants of other viruses, virulent factors of pathogenic bacteria, human disease alleles, and many other genetic targets of interest. Aside from wastewater, our methods could also be useful in assaying other environmental samples or even clinical samples where a polymorphism rich sequence is a desirable target.

4.2. SAM Refiner: Limitations and Future Development

While the outputs of SAM Refiner can be very informative, the program has some limitations, some of which may be overcome in future development. Currently, the greatest limitation is the need for users to be familiar with command line usage. We hope to develop a graphical user interface version to overcome this user hurdle in the future. We also intend to develop SAM Refiner to be available from widely used functional collections such as BioConda (https://bioconda.github.io/accessed on 23 June 2021) and Galaxy (https://usegalaxy.org/accessed on 23 June 2021).

Though SAM Refiner can be used on sequencing not based on amplicons, its usefulness will be more limited in these cases as the relative abundance of sequences and covariants will be calculated based on total reads and not positional coverage. Development to include a mode for whole-genome sequencing or multiple amplicons is in process. The ability to use multiple sequences for a reference may also be added.

The accuracy of the chimera removal algorithms will vary greatly depending on the parameters used and the sample they are being run on. Due to the stochastic nature of chimera generation, and amplification during PCR, and the possible complexity of the original template sequences, samples will sometimes be refractory to chimera removal algorithms. This problem is faced by all programs designed for this purpose. The ability to modify parameters in the algorithms as well as having two algorithms with different approaches to the chimera removal may improve the accuracy the user can achieve with this software. Some samples will, however, always fail to be processed accurately by one or both methods.

Supplementary Materials: The following are available online at https://github.com/degregory/ SR_manuscript/tree/master/Supplementals, Supplementary 1. Example of SAM Refiner's Output for Reporting Unique Sequences, Supplementary 2. Example of SAM Refiner's Output for Reporting Positional NT Calls, Supplementary 3. Example of SAM Refiner's Output for Reporting Insertions and Deletions, Supplementary 4. Example of SAM Refiner's Output for Reporting Covariance, Supplementary 5. Sample Unique Sequences Output With Chimeric Sequences, Supplementary 6. Sample Output of Sequences of SAM Refiner's Chimeras Removed, Supplementary 7. Sample Covariance Output with Chimeric Sequences, Supplementary 8. Sample Passed Sequences Output from the First Part of SAM Refiner's Covariant Deconvolution Method, Supplementary 9. Sample Output of Sequences by SAM Refiner's Covariant Deconvolution Method, Supplementary 10. Assignment of NTD Covariant Sequences to Variants and Lineages, Supplementary 11. Assignment of RBD Covariant Sequences to Variants and Lineages, Supplementary 12. Assignment of S152 Covariant Sequences to Variants and Lineages.

Author Contributions: Conceptualization, M.C.J.; methodology, M.C.J.; software, D.A.G.; validation, D.A.G. and M.C.J.; formal analysis, D.A.G. and M.C.J.; investigation, D.A.G. and M.C.J.; resources, M.C.J. and J.W.; data curation, D.A.G., C.G.W., C.-H.L. and M.C.J.; writing—original draft preparation, D.A.G.; writing—review and editing, D.A.G., J.W., C.G.W. and M.C.J.; visualization, D.A.G.; supervision, M.C.J.; project administration, C.G.W., J.W., and M.C.J.; funding acquisition, J.W. and M.C.J. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for the project was administered by the Missouri Department of Health and Senior Services (DHSS). Research reported in this publication was supported by funding from the Centers for Disease control and the National Institute on Drug Abuse of the National Institutes of Health under award number U01DA053893-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Centers for Disease Control.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw and processed data can be accessed at https://github.com/ degregory/SR_manuscript (accessed on 23 June 2021). Raw sequencing data used in this manuscript and for the MO variant monitoring project will be available pending NCBI processing under BioProject PRJNA748354.

Acknowledgments: We would like to acknowledge Christopher Bottoms for assistance in software development, and the University of Missouri DNA Core for assistance in developing deep sequencing protocols.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data. J.W., affiliated with funder MO DHSS, assisted in editing, and approved publication, of this manuscript.

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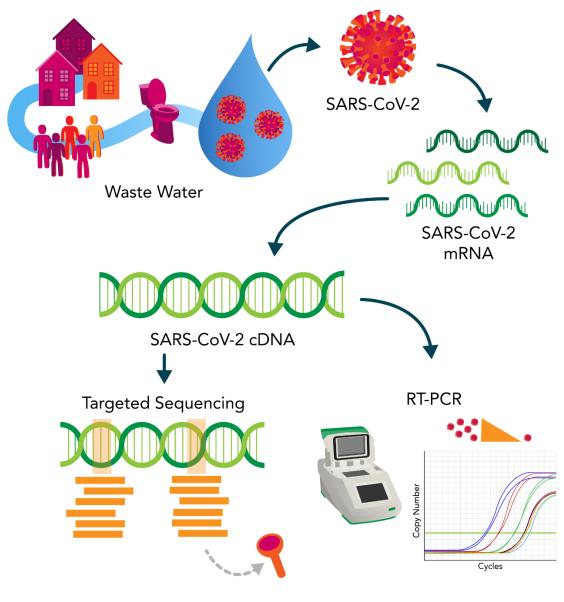
Appendix 5:

Methods

CONCENTRATION METHODS

DEP takes samples of wastewater entering each of City's 14 Wastewater Resource Recovery Facilities (WRRFs), followed by isolating genetic material from SARS-Cov-2. These samples are 24-hour composites, which means that sampling takes place every three hours over that period, samples are combined, and the resulting composite is then tested. Since the pandemic started, composite sampling has become the standard method for sampling for SARS-Cov-2 among wastewater utilities.

Plant influent samples have been analyzed twice every week since April 2020. Between that time and the start of data reporting to DOHMH in September 2020, DEP, along with its academic partners, worked on methods and procedures to ensure reliable and accurate testing. An overview of the sampling and concentrations testing process is shown in the schematic below.



Molly Metz, January 2022

Figure A5.1: Simplified schematic showing the molecular analyses done on the wastewater to monitor for the presence of SARS-CoV2 genetic material (RNA). "RT-PCR" is the name given to the analysis using the N1 Primer to determine SARS-CoV2 RNA concentration. "Targeted sequencing" is the analysis that allows monitoring for indications of variants. Image used with permission, courtesy of Dr. Davida Smyth, Texas &M University.

Samples are put through a three-day workflow that includes pasteurization, solids separation by centrifugation followed by filtration, virus concentration and RNA extraction. Detection and quantitation are performed by RT-PCR. Initially, DEP used the same PCR targets (N1 and N2) that CDC employs in clinical tests throughout the US. Over time, DEP continued with the N1 target only, as it yielded better data quality while reducing burdens on laboratory workload. Quality controls for this work include spiking each sample with the Bovine Coronavirus to assess variability in sample processing; as well as sample duplicates and method blanks. All RNA extracts put through the RT-PCR are analyzed in triplicate. The Limit of Detection is 4,500 copies per liter. Figure A5.2 above shows the sample analysis protocol.

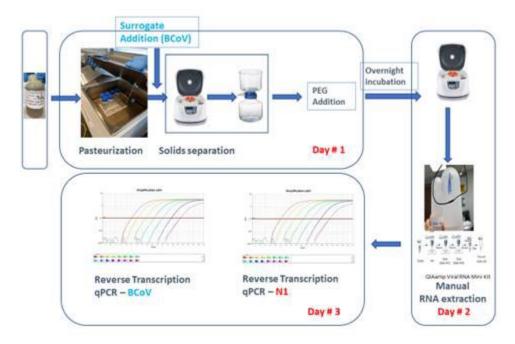


Figure A5.2: Schematic showing the protocol used in the DEP microbiology laboratory to determine the concentration of SARS-CoV2 genetic material (RNA). Image courtesy of DEP.

Measured SARS-CoV-2 concentrations entering each WRRF are converted to viral loads per sewershed population, or the amount of SARS-CoV-2 entering the facility per day per person, adjusted for the quantity of flows through each New York City plant. Results are in turn reported weekly to DOHMH for further analysis and interpretation.

SEQUENCING METHODS

Starting in early 2021, DEP also began to assess sequencing as another method to gain information on SARS-Cov-2 in wastewater. Sequencing involves multiple complex analytical steps, including amplification of selected fragments of the genetic material. The method is designed to target portions of the genome that are prone to mutate (called the receptor binding domain, see Figure A5.3 below). These mutations in turn can be correlated to the variants in circulation. Software is then used to interpret the complex signal obtained from wastewater. "The wastewater sequences are deposited into the GISAID database¹, the most widely used database for SARS-Cov-2 sequences. The deposit sequences were compared to other SARS-Cov-2 sequences, including those from human clinical samples.ⁱ

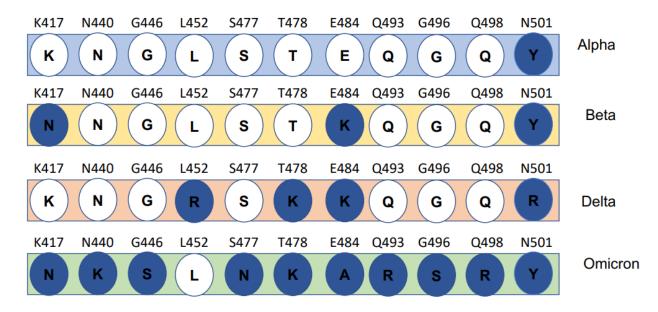


Figure A5.3: Selected amino acids from the receptor binding domain (RBD), a specific region of the SARS-Cov-2 spike protein on the virus's surface. Circles in white represent the original sequence (Wuhan). Circles in dark blue represent mutations. Four-digit codes show the position in the RBD. One-digit letters are codes that correspond to different amino acids.

ⁱ GISAID. 2021. "EpiCov database," <u>https://www.gisaid.org</u> accessed 12/21/2021

Appendix 6

Summary of SARS-CoV-2 wastewater data for New York City's 14 sewersheds for the Omicron wave (November 2021 to January 2022).

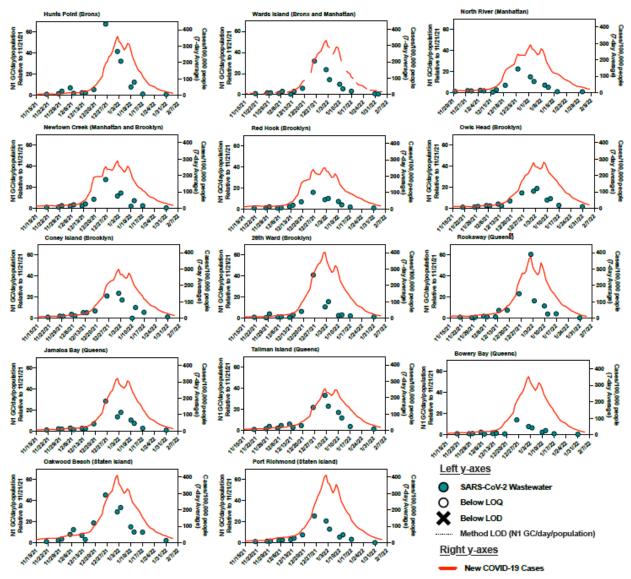


Figure 3.5. Summary of SARS-CoV-2 wastewater data for New York City's 14 sewersheds for the Omicron wave (November 2021 to January 2022). Right y-axis, green circles: SARS-CoV-2 viral loads in influent wastewater normalized by sewershed populations. Left y-axis, red line: 7-day average of new COVID-19 cases/day/100,000 people in the previous 7 days.

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
Sample Date	rescuate	with Name	(N1 Copies/L)	copies per day per	Amotation	estimated
				population)		
8/31/2020		26th Ward	9,858.16		Concentration below Method Limit of Quantification (above Method	290,608
8/31/2020		Bowery Bay	30,509.28		This concentration was obtained using a pooled standard curve	924,695
8/31/2020		Coney Island	7,698.16		Concentration below Method Limit of Quantification (above Method	682,342
8/31/2020		Hunts Point	23,825.06		This concentration was obtained using a pooled standard curve	755,948
8/31/2020		Jamaica Bay	16,001.46		This concentration was obtained using a pooled standard curve	748,737
8/31/2020		Newtown Creek	4,986.60	3,101,240.35	Concentration below Method Limit of Quantification (above Method	1,156,473
8/31/2020		North River			Concentration below Method Limit of Detection; This concentration	658,596
8/31/2020		Oakwood Beach			Concentration below Method Limit of Detection; This concentration	258,731
8/31/2020	9/1/2020	Owls Head			Concentration below Method Limit of Detection; This concentration	906,442
0/04/0000	0 /4 /2020				Concentration below Method Limit of Detection;No signal in 1 out of	226.467
8/31/2020	9/1/2020	Port Richmond			3 RT-qPCR wells, result is obtained by averaging signal from the two	226,167
0/04/0000	0 /4 /2020				Concentration below Method Limit of Detection; No signal in 1 out of	224.020
8/31/2020	9/1/2020		0.400.04	E 045 704 44	3 RT-qPCR wells, result is obtained by averaging signal from the two	224,029
8/31/2020	9/1/2020	Rockaway	9,403.84	5,315,734.41	Concentration below Method Limit of Quantification (above Method	120,539
					Concentration below Method Limit of Quantification (above Method	
8/31/2020		Tallman Island	14,475.95		Limit of Detection);No signal in 1 out of 3 RT-qPCR wells, result is	449,907
8/31/2020		Wards Island	33,930.60	19,776,884.16	This concentration was obtained using a pooled standard curve	1,201,485
9/2/2020		26th Ward			Concentration below Method Limit of Detection; This concentration	290,608
9/2/2020		Bowery Bay			Concentration below Method Limit of Detection; This concentration	924,695
9/2/2020	9/3/2020	Coney Island			analytical issue	682,342
					Concentration below Method Limit of Detection;No signal in 1 out of	
9/2/2020	9/3/2020	Hunts Point			3 RT-qPCR wells, result is obtained by averaging signal from the two	755,948
					Concentration below Method Limit of Quantification (above Method	
9/2/2020		Jamaica Bay	4,609.19	1,747,709.91	Limit of Detection); this sample was analyzed in duplicate. The higher	748,737
9/2/2020		Newtown Creek			Concentration below Method Limit of Detection; This concentration	1,156,473
9/2/2020		North River			analytical issue	658,596
9/2/2020	9/3/2020	Oakwood Beach			analytical issue	258,731
9/2/2020	9/3/2020	Owls Head			analytical issue	906,442
9/2/2020	9/3/2020	Port Richmond			analytical issue	226,167
9/2/2020	9/3/2020	Red Hook	6,119.44	2,481,600.33	Concentration below Method Limit of Quantification (above Method	224,029
9/2/2020	9/3/2020	Rockaway			analytical issue	120,539
9/2/2020	9/3/2020	Tallman Island			possible analytical issue	449,907
9/2/2020	9/3/2020	Wards Island			Concentration below Method Limit of Detection; This concentration	1,201,485
9/6/2020	9/7/2020	26th Ward	10,245.48	5,872,056.12	Concentration below Method Limit of Quantification (above Method	290,608
9/6/2020	9/7/2020	Bowery Bay			Concentration below Method Limit of Detection; This concentration	924,695
					Concentration below Method Limit of Detection; No signal in 1 out of	
9/6/2020	9/7/2020	Coney Island			3 RT-qPCR wells, result is obtained by averaging signal from the two	682,342
9/6/2020	9/7/2020	Hunts Point			possible analytical issue	755,948
9/6/2020	9/7/2020	Jamaica Bay	11,081.45	4,033,784.28	Concentration below Method Limit of Quantification (above Method	748,737
9/6/2020	9/7/2020	Newtown Creek	9,254.13	4,907,133.01	Concentration below Method Limit of Quantification (above Method	1,156,473
9/6/2020	9/7/2020	North River	7,152.69	3,465,700.04	Concentration below Method Limit of Quantification (above Method	658,596
9/6/2020	9/7/2020	Oakwood Beach			Concentration below Method Limit of Detection; This concentration	258,731
9/6/2020	9/7/2020	Owls Head	5,742.73	2,062,479.53	Concentration below Method Limit of Quantification (above Method	906,442
					Concentration below Method Limit of Detection; No signal in 1 out of	
9/6/2020	9/7/2020	Port Richmond			3 RT-qPCR wells, result is obtained by averaging signal from the two	226,167
					Concentration below Method Limit of Quantification (above Method	
9/6/2020	9/7/2020	Red Hook	10,396.87	3,689,184.68	Limit of Detection); this sample was analyzed in duplicate. The	224,029
9/6/2020	9/7/2020		,	, ,	Concentration below Method Limit of Detection; This concentration	120,539
					Concentration below Method Limit of Detection; No signal is 2 out of	
9/6/2020	9/7/2020	Tallman Island			3 RT-qPCR wells, result in obtained by averaging signal from the	449,907
9/6/2020		Wards Island			analytical issue	1,201,485
9/8/2020		26th Ward	12,631.00	7.403.813.15	Concentration below Method Limit of Quantification (above Method	290,608
9/8/2020		Bowery Bay	24,097.61		This concentration was obtained using a pooled standard curve	924,695
9/8/2020		Coney Island	1,096,550.51		This concentration was obtained using a pooled standard curve	682,342
9/8/2020		Hunts Point	40,075.54		This concentration was obtained using a pooled standard curve	755,948
9/8/2020		Jamaica Bay	31,015.23		This concentration was obtained using a pooled standard curve	748,737
9/8/2020		Newtown Creek	7,773.81		Concentration below Method Limit of Quantification (above Method	1,156,473
9/8/2020		North River	10,505.44		Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method	658,596
5/ 5/ 2020	5, 5, 2020		10,505.44	5,700,431.31	Concentration below Method Limit of Quantification (above Method	050,550
9/8/2020	9/9/2020	Oakwood Beach	13,659.49	4 888 271 25	Limit of Detection); this sample was analyzed in duplicate. The	258,731
9/8/2020		Owls Head	21,841.02		This concentration was obtained using a pooled standard curve	906,442
9/8/2020		Port Richmond	21,841.02 26,821.95		This concentration was obtained using a pooled standard curve	226,167
9/8/2020	9/9/2020		12,116.13		Concentration below Method Limit of Quantification (above Method	226,167
9/8/2020					Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method	
	9/9/2020		6,841.80		· · · · · ·	120,539
9/8/2020		Tallman Island	18,992.56		This concentration was obtained using a pooled standard curve	449,907
9/8/2020		Wards Island	26,973.81	15,807,012.61	This concentration was obtained using a pooled standard curve	1,201,485
9/13/2020	9/14/2020			44 004 007 00	possible analytical issue	290,608
9/13/2020		Bowery Bay	32,391.92		This concentration was obtained using a pooled standard curve	924,695
9/13/2020		Coney Island	429,574.30		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
9/13/2020		Hunts Point	12,456.79		Concentration below Method Limit of Quantification (above Method	755,948
9/13/2020		Jamaica Bay	11,904.99	4,514,128.48	Concentration below Method Limit of Quantification (above Method	748,737
9/13/2020	9/14/2020	Newtown Creek			Concentration below Method Limit of Detection; This concentration	1,156,473

			Concentration CARC	Per capita SARS-		Donulation
Sample Date	Test date	WRRF Name	Concentration SARS- CoV-2 gene target	CoV-2 load (N1	Annotation	Population Served,
Sample Date	Test date	WINE Name	(N1 Copies/L)	copies per day per	Amotation	estimated
0 (40 /2020	0/44/2022		(population)		
9/13/2020		North River	5,761.77	2 101 764 04	Concentration below Method Limit of Detection; This concentration Concentration below Method Limit of Quantification (above Method	658,596
9/13/2020 9/13/2020	9/14/2020	Oakwood Beach	5,761.77	2,191,764.94	Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Detection; This concentration	258,731 906,442
9/13/2020		Port Richmond			Concentration below Method Limit of Detection; This concentration	226,167
9/13/2020	9/14/2020		11,411.37	4,434,803.84		220,107
9/13/2020	9/14/2020		11,411.57	-,-5-,005.0+	Concentration below Method Limit of Detection; This concentration	120,539
9/13/2020		Tallman Island			possible analytical issue	449,907
9/13/2020		Wards Island	9,990.02	5,822,808.05		1,201,485
9/15/2020	9/16/2020	26th Ward	13,270.46	7,778,644.12	Concentration below Method Limit of Quantification (above Method	290,608
9/15/2020	9/16/2020	Bowery Bay	24,617.06	8,666,605.11	This concentration was obtained using a pooled standard curve	924,695
9/15/2020	9/16/2020	Coney Island	34,718.49	15,215,936.94	This concentration was obtained using a pooled standard curve	682,342
9/15/2020	9/16/2020	Hunts Point	26,605.12	15,454,119.27	This concentration was obtained using a pooled standard curve	755,948
9/15/2020		Jamaica Bay	12,586.86		Concentration below Method Limit of Quantification (above Method	748,737
9/15/2020	9/16/2020	Newtown Creek	18,721.43	10,601,385.61	This concentration was obtained using a pooled standard curve	1,156,473
					Concentration below Method Limit of Quantification (above Method	
9/15/2020		North River	6,281.49		Limit of Detection); this sample was analyzed in duplicate. The	658,596
9/15/2020		Oakwood Beach	7,234.00		Concentration below Method Limit of Quantification (above Method	258,731
9/15/2020		Owls Head	68,233.78		This concentration was obtained using a pooled standard curve	906,442
9/15/2020 9/15/2020	9/16/2020	Port Richmond Red Hook	6,250.51 6,979.92		Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method	226,167
9/15/2020	9/16/2020		0,373.92	2,712,007.00	analytical issue	120,539
9/15/2020		Tallman Island	6,696.78	2.817.254.21	Concentration below Method Limit of Quantification (above Method	449,907
9/15/2020		Wards Island	7,994.37		Concentration below Method Limit of Quantification (above Method	1,201,485
9/20/2020	9/21/2020		10,938.55	, ,	Concentration below Method Limit of Quantification (above Method	290,608
9/20/2020		Bowery Bay	36,117.23		This concentration was obtained using a pooled standard curve	924,695
9/20/2020	9/21/2020	Coney Island	469,838.05	211,127,118.93	This concentration was obtained using a pooled standard curve	682,342
9/20/2020	9/21/2020	Hunts Point	14,384.01	8,571,324.16	Concentration below Method Limit of Quantification (above Method	755,948
9/20/2020	9/21/2020	Jamaica Bay	20,301.08	7,697,752.98	This concentration was obtained using a pooled standard curve	748,737
9/20/2020		Newtown Creek	21,127.10		This concentration was obtained using a pooled standard curve	1,156,473
9/20/2020		North River	25,101.61		This concentration was obtained using a pooled standard curve	658,596
9/20/2020		Oakwood Beach	16,309.86		This concentration was obtained using a pooled standard curve	258,731
9/20/2020	9/21/2020		69,988.93		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
9/20/2020		Port Richmond	21,533.82	7,893,122.50	This concentration was obtained using a pooled standard curve	226,167
9/20/2020 9/20/2020	9/21/2020 9/21/2020		15,913.64	10 /0/ 706 01	Concentration below Method Limit of Detection; This concentration This concentration was obtained using a pooled standard curve	224,029
9/20/2020		Tallman Island	13,513.04	10,494,790.91	possible analytical issue	449,907
9/20/2020		Wards Island	31,677.17	17 265 813 55	This concentration was obtained using a pooled standard curve	1,201,485
9/22/2020	9/23/2020		21,157.94		This concentration was obtained using a pooled standard curve	290,608
9/22/2020		Bowery Bay	7,327.50		Concentration below Method Limit of Quantification (above Method	924,695
9/22/2020	9/23/2020	Coney Island	6,862.74	3,159,994.55	Concentration below Method Limit of Quantification (above Method	682,342
9/22/2020	9/23/2020	Hunts Point	7,418.38	4,271,966.91	Concentration below Method Limit of Quantification (above Method	755,948
9/22/2020	9/23/2020	Jamaica Bay	117,174.31	42,060,495.56	This concentration was obtained using a pooled standard curve	748,737
9/22/2020	9/23/2020	Newtown Creek	10,458.67	6,401,704.44	Concentration below Method Limit of Quantification (above Method	1,156,473
9/22/2020		North River			Concentration below Method Limit of Detection; This concentration	658,596
9/22/2020		Oakwood Beach	38,644.39	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	258,731
9/22/2020	9/23/2020	Owls Head	25,625.37	9,203,255.04	This concentration was obtained using a pooled standard curve	906,442
0/00/000	0/22/222	De et Di l			Concentration below Method Limit of Detection; No signal is 2 out of	
9/22/2020		Port Richmond			3 RT-qPCR wells, result in obtained by averaging signal from the	226,167
9/22/2020 9/22/2020	9/23/2020			<u> </u>	Concentration below Method Limit of Detection; This concentration Concentration below Method Limit of Detection; This concentration	224,029
9/22/2020	9/23/2020	Tallman Island	6,569.98	2 652 254 01	Concentration below Method Limit of Detection; This concentration Concentration below Method Limit of Quantification (above Method	449,907
9/22/2020		Wards Island	0,303.98	2,000,004.01	Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Detection; This concentration	1,201,485
9/22/2020	9/30/2020		49,671.85	31.056 815 07	original RT-qPCR (9/28/2020) failed, RT-qPCR repeated;This	290,608
9/27/2020		Bowery Bay	35,872.18		This concentration was obtained using a pooled standard curve	924,695
9/27/2020		Coney Island	43,437.57		This concentration was obtained using a pooled standard curve	682,342
9/27/2020		Hunts Point	19,794.83		This concentration was obtained using a pooled standard curve	755,948
9/27/2020		Jamaica Bay	19,076.89		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
9/27/2020		Newtown Creek	25,514.20		This concentration was obtained using a pooled standard curve	1,156,473
9/27/2020		North River	26,999.15	12,880,180.82	original RT-qPCR (9/28/2020) failed, RT-qPCR repeated; This	658,596
9/27/2020		Oakwood Beach	16,361.20		No signal in 1 out of 3 RT-qPCR wells, result is obtained by averaging	258,731
9/27/2020	9/30/2020		131,091.40		original RT-qPCR (9/28/2020) failed, RT-qPCR repeated; This	906,442
9/27/2020		Port Richmond	43,453.95		This concentration was obtained using a pooled standard curve	226,167
9/27/2020	9/28/2020		5,431.56	2,110,868.03	Concentration below Method Limit of Quantification (above Method	224,029
9/27/2020	9/28/2020		10 5 45	4 610 011	analytical issue	120,539
9/27/2020		Tallman Island	10,545.71	4,613,911.35	Concentration below Method Limit of Quantification (above Method	449,907
0/27/2020		Wards Island			analytical issue	1,201,485
9/27/2020	0/20/2020				This result is not useable (because associated with contaminated	290,608
9/29/2020	9/30/2020					
9/29/2020 9/29/2020	9/30/2020	Bowery Bay			This result is not useable (because associated with contaminated This result is not useable (because associated with contaminated	924,695
9/29/2020 9/29/2020 9/29/2020	9/30/2020 9/30/2020	Bowery Bay Coney Island			This result is not useable (because associated with contaminated	682,342
9/29/2020 9/29/2020	9/30/2020 9/30/2020 9/30/2020	Bowery Bay				

Sample Date	Test date	WRRF Name	Concentration SARS- CoV-2 gene target (N1 Copies/L)	Per capita SARS- CoV-2 load (N1 copies per day per population)	Annotation	Population Served, estimated
9/29/2020	9/30/2020	North River		population	This result is not useable (because associated with contaminated	658,596
9/29/2020		Oakwood Beach			This result is not useable (because associated with contaminated	258,731
9/29/2020	9/30/2020	Owls Head			This result is not useable (because associated with contaminated	906,442
9/29/2020	9/30/2020	Port Richmond			This result is not useable (because associated with contaminated	226,167
9/29/2020	9/30/2020	Red Hook			This result is not useable (because associated with contaminated	224,029
9/29/2020	9/30/2020	Rockaway			This result is not useable (because associated with contaminated	120,539
9/29/2020	9/30/2020	Tallman Island			This result is not useable (because associated with contaminated	449,907
9/29/2020		Wards Island			This result is not useable (because associated with contaminated	1,201,485
10/4/2020	10/5/2020				This result is not useable (because associated with contaminated	290,608
10/4/2020		Bowery Bay			This result is not useable (because associated with contaminated	924,695
10/4/2020		Coney Island			This result is not useable (because associated with contaminated	682,342
10/4/2020		Hunts Point			This result is not useable (because associated with contaminated	755,948
10/4/2020		Jamaica Bay			This result is not useable (because associated with contaminated	748,737
10/4/2020		Newtown Creek			This result is not useable (because associated with contaminated	1,156,473
10/4/2020		North River			This result is not useable (because associated with contaminated	658,596
10/4/2020		Oakwood Beach			This result is not useable (because associated with contaminated	258,731
10/4/2020	10/5/2020				This result is not useable (because associated with contaminated	906,442
10/4/2020		Port Richmond			This result is not useable (because associated with contaminated	226,167
10/4/2020	10/5/2020				This result is not useable (because associated with contaminated	224,029
10/4/2020	10/5/2020				This result is not useable (because associated with contaminated	120,539
10/4/2020		Tallman Island			This result is not useable (because associated with contaminated	449,907
10/4/2020		Wards Island	20.645.02	47 745 540 74	This result is not useable (because associated with contaminated	1,201,485
10/6/2020	10/7/2020		29,615.93		This concentration was obtained using a pooled standard curve	290,608
10/6/2020		Bowery Bay	70,945.13		This concentration was obtained using a pooled standard curve	924,695
10/6/2020		Coney Island	183,398.46		This concentration was obtained using a pooled standard curve	682,342
10/6/2020		Hunts Point	26,635.01		This concentration was obtained using a pooled standard curve	755,948
10/6/2020 10/6/2020		Jamaica Bay	33,369.87		This concentration was obtained using a pooled standard curve	748,737
10/6/2020		Newtown Creek North River	36,693.85 20,865.38		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473 658,596
10/6/2020		Oakwood Beach	78,739.14		This concentration was obtained using a pooled standard curve	258,731
10/6/2020	10/7/2020		147,560.16		This concentration was obtained using a pooled standard curve	906,442
10/6/2020		Port Richmond	35,550.76		This concentration was obtained using a pooled standard curve	226,167
10/6/2020	10/7/2020		22,251.71		This concentration was obtained using a pooled standard curve	220,107
10/6/2020	10/7/2020		26,730.86		This concentration was obtained using a pooled standard curve	120,539
10/6/2020		Tallman Island	14,856.23		Concentration below Method Limit of Quantification (above Method	449,907
10/6/2020		Wards Island	29,198.78		This concentration was obtained using a pooled standard curve	1,201,485
10/11/2020	10/12/2020		14,416.69		Concentration below Method Limit of Quantification (above Method	290,608
10/11/2020			84,621.88		This concentration was obtained using a pooled standard curve	924,695
10/11/2020		Coney Island	42,079.63		This concentration was obtained using a pooled standard curve	682,342
	10/12/2020		21,429.87		This concentration was obtained using a pooled standard curve	755,948
	10/12/2020		39,777.67		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
		Newtown Creek	17,270.90		This concentration was obtained using a pooled standard curve	1,156,473
	10/12/2020		8,447.89	4,321,474.18	Concentration below Method Limit of Quantification (above Method	658,596
		Oakwood Beach	53,358.32	18,501,840.66	This concentration was obtained using a pooled standard curve	258,731
	10/12/2020		152,981.08		This concentration was obtained using a pooled standard curve	906,442
10/11/2020	10/12/2020	Port Richmond	37,725.23	14,522,569.87	This concentration was obtained using a pooled standard curve	226,167
10/11/2020	10/12/2020	Red Hook			Concentration below Method Limit of Detection; This concentration	224,029
10/11/2020	10/12/2020	Rockaway	27,319.92	15,443,200.66	This concentration was obtained using a pooled standard curve	120,539
10/11/2020	10/12/2020	Tallman Island	15,855.46		This concentration was obtained using a pooled standard curve	449,907
10/11/2020	10/12/2020	Wards Island	15,207.78	8,145,342.78	This concentration was obtained using a pooled standard curve	1,201,485
	10/14/2020		34,238.31		No signal in 1 out of 3 RT-qPCR wells, result is obtained by averaging	290,608
	10/14/2020		59,245.52	, ,	This concentration was obtained using a pooled standard curve	924,695
	10/14/2020		351,793.01		This concentration was obtained using a pooled standard curve	682,342
	10/14/2020		46,161.52		This concentration was obtained using a pooled standard curve	755,948
	10/14/2020		54,983.61		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	53,262.04		This concentration was obtained using a pooled standard curve	1,156,473
10/13/2020			25,626.02		This concentration was obtained using a pooled standard curve	658,596
10/13/2020		Oakwood Beach	43,399.26		This concentration was obtained using a pooled standard curve	258,731
10/13/2020			118,386.14		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
10/13/2020		Port Richmond	50,837.98		This concentration was obtained using a pooled standard curve	226,167
	10/14/2020		32,808.85		This concentration was obtained using a pooled standard curve	224,029
10/13/2020		,	26,455.15		This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	13,693.00		Concentration below Method Limit of Quantification (above Method	449,907
10/13/2020	10/14/2020	Wards Island	52,346.77	33,809,465.04	This concentration was obtained using a pooled standard curve	1,201,485
10/10/202	10/100/1000-	act w		o 400	Concentration below Method Limit of Quantification (above Method	200
	10/19/2020		14,923.01		Limit of Detection);associated method blank contaminated; singal in	290,608
	10/19/2020		99,453.67		associated method blank contaminated; singal in the sample	924,695
	10/19/2020		66,257.25		associated method blank contaminated; singal in the sample	682,342
	10/19/2020		64,988.62		associated method blank contaminated; singal in the sample	755,948
	10/19/2020		33,264.88		associated method blank contaminated; singal in the sample	748,737
10/18/2020 10/18/2020		Newtown Creek	154,572.89		associated method blank contaminated; singal in the sample	1,156,473
10/18/20201	10/19/2020	NUT LIT KIVET	37,483.35	19,389,864.00	associated method blank contaminated; singal in the sample	658,596

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1 copies per day per	Annotation	Served,
			(N1 Copies/L)	population)		estimated
10/18/2020	10/19/2020	Oakwood Beach	45,438.79		associated method blank contaminated; singal in the sample	258,73
10/18/2020	10/19/2020	Owls Head	196,361.13	71,342,393.83	associated method blank contaminated; singal in the sample	906,44
10/18/2020	10/19/2020	Port Richmond	31,139.55	13,029,749.82	associated method blank contaminated; singal in the sample	226,16
					this sample was analyzed in duplicate. The higher of the 2 results is	
10/18/2020	10/19/2020	Red Hook	46,882.50	16,635,611.23	reported;associated method blank contaminated; singal in the	224,02
40/40/2020	40/40/2020		6 600 70	4 500 007 54	Concentration below Method Limit of Quantification (above Method	400 50
, ,	10/19/2020	,	6,633.79	, ,	Limit of Detection);associated method blank contaminated; singal in	120,53
10/18/2020	10/19/2020	Tallman Island	15,398.67	8,291,885.77	associated method blank contaminated; singal in the sample Concentration below Method Limit of Quantification (above Method	449,90
10/18/2020	10/19/2020	Wards Island	11,261.89	6 173 837 82	Limit of Detection);associated method blank contaminated; singal in	1,201,48
, ,	10/21/2020		44,452.40	, ,	This concentration was obtained using a pooled standard curve	290,60
	10/21/2020		110,929.62	,,	This concentration was obtained using a pooled standard curve	924,69
		Coney Island	62,151.08		This concentration was obtained using a pooled standard curve	682,34
10/20/2020	10/21/2020	Hunts Point	58,351.67	32,433,759.02	This concentration was obtained using a pooled standard curve	755,94
10/20/2020	10/21/2020	Jamaica Bay	96,496.74	35,979,763.79	This concentration was obtained using a pooled standard curve	748,73
10/20/2020	10/21/2020	Newtown Creek	134,099.38	75,497,513.96	this sample was analyzed in duplicate. The higher of the 2 results is	1,156,47
10/20/2020	10/21/2020	North River	45,316.47	23,962,809.71	This concentration was obtained using a pooled standard curve	658,59
		Oakwood Beach	121,482.39		This concentration was obtained using a pooled standard curve	258,73
	10/21/2020		461,895.23		This concentration was obtained using a pooled standard curve	906,44
		Port Richmond	96,000.91		This concentration was obtained using a pooled standard curve	226,16
	10/21/2020		50,261.43	, ,	This concentration was obtained using a pooled standard curve	224,02
	10/21/2020		68,922.93		This concentration was obtained using a pooled standard curve	120,53
		Tallman Island	56,397.33		This concentration was obtained using a pooled standard curve	449,90
	10/21/2020	Wards Island	76,683.79 41,850.92	, ,	This concentration was obtained using a pooled standard curve	1,201,48
	10/26/2020		61,576.39		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,69
		Coney Island	66,256.32		This concentration was obtained using a pooled standard curve	682,34
	10/26/2020		67,852.71		This concentration was obtained using a pooled standard curve	755,94
			44,451.69		This concentration was obtained using a pooled standard curve	748,73
		Newtown Creek	69,899.18		This concentration was obtained using a pooled standard curve	1,156,47
			43,270.95		this sample was analyzed in duplicate. The higher of the 2 results is	658,59
10/25/2020	10/26/2020	Oakwood Beach	96,830.92	35,134,209.30	This concentration was obtained using a pooled standard curve	258,73
10/25/2020	10/26/2020	Owls Head	144,875.26	53,241,441.40	This concentration was obtained using a pooled standard curve	906,44
10/25/2020	10/26/2020	Port Richmond	61,299.22	22,879,339.38	This concentration was obtained using a pooled standard curve	226,16
	10/26/2020		18,853.39	6,689,866.99	This concentration was obtained using a pooled standard curve	224,02
	10/26/2020		32,227.86		This concentration was obtained using a pooled standard curve	120,53
		Tallman Island	45,520.84	19,150,073.64		449,90
		Wards Island	51,040.02		This concentration was obtained using a pooled standard curve	1,201,48
	10/28/2020		48,828.00		This concentration was obtained using a pooled standard curve	290,60
	10/28/2020	Coney Island	99,144.77 56,151.07		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,69
	10/28/2020		168,637.56		This concentration was obtained using a pooled standard curve	755,94
			66,011.32		This concentration was obtained using a pooled standard curve	748,73
		Newtown Creek	79,361.02	, ,	This concentration was obtained using a pooled standard curve	1,156,47
	10/28/2020		43,663.82		This concentration was obtained using a pooled standard curve	658,59
		Oakwood Beach	79,769.06		This concentration was obtained using a pooled standard curve	258,73
	10/28/2020		182,152.88	61,616,064.88	This concentration was obtained using a pooled standard curve	906,44
10/27/2020	10/28/2020	Port Richmond	115,748.89	43,783,345.56	This concentration was obtained using a pooled standard curve	226,16
10/27/2020	10/28/2020	Red Hook	134,228.54	49,897,205.28	This concentration was obtained using a pooled standard curve	224,02
10/27/2020	10/28/2020	Rockaway	22,386.14	12,654,268.30	This concentration was obtained using a pooled standard curve	120,53
		Tallman Island	41,080.16		this sample was analyzed in duplicate. The higher of the 2 results is	449,90
10/27/2020		Wards Island	92,869.86	, ,	This concentration was obtained using a pooled standard curve	1,201,48
11/1/2020	11/2/2020		39,947.61		This concentration was obtained using a pooled standard curve	290,60
11/1/2020		Bowery Bay	108,919.74	62,869,516.21		924,69
11/1/2020		Coney Island	60,552.85	30,569,391.84		682,34
11/1/2020		Hunts Point	58,332.55 63,150.67	46,443,945.21		755,94
11/1/2020 11/1/2020		Jamaica Bay Newtown Creek	65,802.07	31,607,984.81	This concentration was obtained using a pooled standard curve	748,73
11/1/2020		North River	42,397.17	25,343,348.82		658,59
11/1/2020		Oakwood Beach	72,072.87	34,059,541.82		258,73
11/1/2020	11/2/2020		194,244.99		this sample was analyzed in duplicate. The higher of the 2 results is	906,44
11/1/2020		Port Richmond	70,979.50		This concentration was obtained using a pooled standard curve	226,16
11/1/2020	11/2/2020		19,628.41		This concentration was obtained using a pooled standard curve	224,02
11/1/2020	11/3/2020		11,462.71		Concentration below Method Limit of Quantification (above Method	120,53
11/1/2020		Tallman Island	27,509.85		This concentration was obtained using a pooled standard curve	449,90
11/1/2020		Wards Island	105,411.63		This concentration was obtained using a pooled standard curve	1,201,48
11/1/2020		achte Manuel	91,624.32	58,480,654.03	Sample processing method slightly different due to supply chain	290,60
	11/4/2020	26th Ward	51,02 1102			
11/1/2020 11/3/2020 11/3/2020	11/4/2020 11/4/2020	Bowery Bay	135,502.25		Sample processing method slightly different due to supply chain	924,69
11/1/2020 11/3/2020 11/3/2020 11/3/2020	11/4/2020 11/4/2020 11/4/2020	Bowery Bay Coney Island	135,502.25 29,012.22	12,393,173.15	Sample processing method slightly different due to supply chain	682,34
11/1/2020 11/3/2020 11/3/2020	11/4/2020 11/4/2020 11/4/2020 11/4/2020	Bowery Bay	135,502.25	12,393,173.15 31,564,163.36		924,69 682,34 755,94 748,73

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
11/3/2020	11/4/2020	North River	101,754.59		Sample processing method slightly different due to supply chain	658,596
11/3/2020		Oakwood Beach	72,077.73		Sample processing method slightly different due to supply chain	258,731
					this sample was analyzed in duplicate. The higher of the 2 results is	
11/3/2020	11/4/2020	Owls Head	134,881.86	47,879,033.23	reported;Sample processing method slightly different due to supply	906,442
11/3/2020		Port Richmond	215,070.43		Sample processing method slightly different due to supply chain	226,167
11/3/2020	11/4/2020		103,836.31		Sample processing method slightly different due to supply chain	224,029
11/3/2020	11/4/2020		24,479.66		Sample processing method slightly different due to supply chain	120,539
11/3/2020		Tallman Island	69,395.52	, ,	Sample processing method slightly different due to supply chain	449,907
11/3/2020 11/8/2020	11/4/2020	Wards Island	91,321.71 111,506.22		Sample processing method slightly different due to supply chain This concentration was obtained using a pooled standard curve	1,201,485 290,608
11/8/2020		Bowery Bay	386,043.28		This concentration was obtained using a pooled standard curve	924,695
11/8/2020		Coney Island	276,679.52	, ,	This concentration was obtained using a pooled standard curve	682,342
11/8/2020		Hunts Point	165,158.21		This concentration was obtained using a pooled standard curve	755,948
11/8/2020	11/9/2020	Jamaica Bay	287,713.65		This concentration was obtained using a pooled standard curve	748,737
11/8/2020	11/9/2020	Newtown Creek	236,669.41	125,497,356.31	this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
11/8/2020	11/9/2020	North River	213,595.44	99,442,226.97	This concentration was obtained using a pooled standard curve	658,596
11/8/2020		Oakwood Beach	262,695.12		This concentration was obtained using a pooled standard curve	258,731
11/8/2020	11/9/2020		305,014.03	,. ,	This concentration was obtained using a pooled standard curve	906,442
11/8/2020		Port Richmond	320,488.62		This concentration was obtained using a pooled standard curve	226,167
11/8/2020 11/8/2020	11/9/2020 11/9/2020		55,384.49		This concentration was obtained using a pooled standard curve	224,029
11/8/2020		Tallman Island	52,498.63 105,620.46		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
11/8/2020		Wards Island	208,294.53		This concentration was obtained using a pooled standard curve	1,201,485
11/10/2020			147,288.60		This concentration was obtained using a pooled standard curve	290,608
11/10/2020			303,466.33		This concentration was obtained using a pooled standard curve	924,695
11/10/2020	11/11/2020	Coney Island	120,472.29	50,125,559.94	This concentration was obtained using a pooled standard curve	682,342
11/10/2020	11/17/2020	Hunts Point	16,361.12	9,421,757.05	This concentration was obtained using a pooled standard curve	755,948
11/10/2020			287,993.16	107,745,098.95	this sample was analyzed in duplicate. The higher of the 2 results is	748,737
11/10/2020		Newtown Creek	161,538.18		This concentration was obtained using a pooled standard curve	1,156,473
11/10/2020			199,710.26		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	178,122.55		This concentration was obtained using a pooled standard curve	258,731
	11/11/2020	Port Richmond	284,435.33 413,360.04		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
	11/11/2020		236,739.08		This concentration was obtained using a pooled standard curve	220,107
	11/11/2020		14,839.44		Concentration below Method Limit of Quantification (above Method	120,539
		Tallman Island	222,103.90		This concentration was obtained using a pooled standard curve	449,907
11/10/2020	11/11/2020	Wards Island	209,219.40	111,399,496.91	This concentration was obtained using a pooled standard curve	1,201,485
					this sample was analyzed in duplicate. The higher of the 2 results is	
	11/16/2020		62,442.24	, ,	reported;Sample processing method slightly different due to supply	290,608
	11/16/2020		40,556.93		Sample processing method slightly different due to supply chain	924,695
	11/16/2020		43,911.25		Sample processing method slightly different due to supply chain	682,342
11/15/2020			119,184.21 478,599.35		Sample processing method slightly different due to supply chain Sample processing method slightly different due to supply chain	755,948 748,737
11/15/2020		Newtown Creek	58,181.98		Sample processing method slightly different due to supply chain	1,156,473
	11/16/2020		20,653.53		Sample processing method slightly different due to supply chain	658,596
		Oakwood Beach	68,241.82		Sample processing method slightly different due to supply chain	258,731
	11/16/2020		86,606.16		Sample processing method slightly different due to supply chain	906,442
11/15/2020	11/16/2020	Port Richmond	42,601.40	26,382,095.67	Sample processing method slightly different due to supply chain	226,167
	11/16/2020		24,964.60		Sample processing method slightly different due to supply chain	224,029
	11/16/2020		22,969.70		Sample processing method slightly different due to supply chain	120,539
		Tallman Island	44,121.69		Sample processing method slightly different due to supply chain	449,907
	11/16/2020 11/18/2020	Wards Island 26th Ward	65,174.52 119,328.80		Sample processing method slightly different due to supply chain This concentration was obtained using a pooled standard curve	1,201,485 290,608
	11/18/2020		222,628.64		This concentration was obtained using a pooled standard curve	924,695
	11/18/2020		91,225.17		This concentration was obtained using a pooled standard curve	682,342
	11/18/2020		105,497.48		This concentration was obtained using a pooled standard curve	755,948
	11/18/2020		97,378.44		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	147,726.47	82,685,973.63	This concentration was obtained using a pooled standard curve	1,156,473
	11/18/2020		104,129.22		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	88,654.35		This concentration was obtained using a pooled standard curve	258,731
	11/18/2020		215,077.40		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
		Port Richmond	171,878.54		This concentration was obtained using a pooled standard curve	226,167
	11/18/2020 11/18/2020		101,701.67 53,169.20		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	224,029 120,539
		коскаway Tallman Island	65,202.94		This concentration was obtained using a pooled standard curve	449,907
11/17/2020		Wards Island	135,932.26		This concentration was obtained using a pooled standard curve	1,201,485
	11/23/2020		253,115.70		This concentration was obtained using a pooled standard curve	290,608
11/22/2020			314,744.17		this sample was analyzed in duplicate. The higher of the 2 results is	924,695
11/22/2020	11/23/2020	Coney Island	130,981.22		This concentration was obtained using a pooled standard curve	682,342
	11/23/2020		227,855.45		This concentration was obtained using a pooled standard curve	755,948
11/22/2020	11/23/2020	Jamaica Bay	283,109.41		This concentration was obtained using a pooled standard curve	748,737
11/22/2020		Newtown Creek	138,876.40		This concentration was obtained using a pooled standard curve	1,156,473

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1 copies per day per	Annotation	Served,
			(N1 Copies/L)	population)		estimated
11/22/2020	11/23/2020	North River	264,318.09		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	69,521.45	26,750,940.29	This concentration was obtained using a pooled standard curve	258,731
	11/23/2020		134,555.19		This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	308,753.46		This concentration was obtained using a pooled standard curve	226,167
	11/23/2020 11/23/2020		199,911.38 24,258.46		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	224,029 120,539
		Tallman Island	181,503.07		This concentration was obtained using a pooled standard curve	449,907
		Wards Island	251,473.94		This concentration was obtained using a pooled standard curve	1,201,485
	11/25/2020		273,701.11		This concentration was obtained using a pooled standard curve	290,608
11/24/2020	11/25/2020	Bowery Bay	341,593.26	114,666,763.65	This concentration was obtained using a pooled standard curve	924,695
		Coney Island	150,759.22		This concentration was obtained using a pooled standard curve	682,342
	11/25/2020		270,036.62		This concentration was obtained using a pooled standard curve	755,948
	11/25/2020	Jamaica Bay Newtown Creek	417,738.35 166,089.56		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737 1,156,473
	11/25/2020		157,853.09	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	658,596
		Oakwood Beach	373,314.38		This concentration was obtained using a pooled standard curve	258,731
	11/25/2020		358,968.08		This concentration was obtained using a pooled standard curve	906,442
11/24/2020	11/25/2020	Port Richmond	428,793.10	165,066,640.48	This concentration was obtained using a pooled standard curve	226,167
	11/25/2020		163,781.47		This concentration was obtained using a pooled standard curve	224,029
	11/25/2020		70,570.53		This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	73,551.36 217,922.55		This concentration was obtained using a pooled standard curve	449,907
	11/25/2020	Wards Island 26th Ward	217,922.55		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
	11/30/2020		523,705.50		This concentration was obtained using a pooled standard curve	924,695
		Coney Island	949,804.06	, ,	This concentration was obtained using a pooled standard curve	682,342
11/29/2020	11/30/2020	Hunts Point	399,406.77	242,003,585.01	This concentration was obtained using a pooled standard curve	755,948
	11/30/2020		138,044.85		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	387,155.41		This concentration was obtained using a pooled standard curve	1,156,473
	11/30/2020		189,466.49		This concentration was obtained using a pooled standard curve	658,596
	11/30/2020	Oakwood Beach	379,348.33 122,381.64		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
		Port Richmond	361,629.25		This concentration was obtained using a pooled standard curve	226,167
	11/30/2020		155,513.90		this sample was analyzed in duplicate. The higher of the 2 results is	224,029
11/29/2020	11/30/2020	Rockaway	85,444.37	50,982,650.89	This concentration was obtained using a pooled standard curve	120,539
11/29/2020		Tallman Island	375,203.84	157,843,771.29	This concentration was obtained using a pooled standard curve	449,907
		Wards Island	231,169.19		This concentration was obtained using a pooled standard curve	1,201,485
12/1/2020 12/1/2020		26th Ward Bowery Bay	155,000.10 517,174.76		This concentration was obtained using a pooled standard curve	290,608 924,695
12/1/2020		Coney Island	251,992.99		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	682,342
12/1/2020		Hunts Point	188,012.87		This concentration was obtained using a pooled standard curve	755,948
12/1/2020		Jamaica Bay	462,096.16		This concentration was obtained using a pooled standard curve	748,737
12/1/2020	12/2/2020	Newtown Creek	117,963.94	68,730,055.69	This concentration was obtained using a pooled standard curve	1,156,473
12/1/2020		North River	131,790.23		This concentration was obtained using a pooled standard curve	658,596
12/1/2020		Oakwood Beach	132,923.61		This concentration was obtained using a pooled standard curve	258,731
12/1/2020 12/1/2020		Owls Head Port Richmond	485,986.93 753,152.84		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
12/1/2020			107,996.67		This concentration was obtained using a pooled standard curve	226,167
12/1/2020			49,915.26		This concentration was obtained using a pooled standard curve	120,539
12/1/2020		Tallman Island	162,727.48		This concentration was obtained using a pooled standard curve	449,907
12/1/2020		Wards Island	297,154.61		This concentration was obtained using a pooled standard curve	1,201,485
12/6/2020		26th Ward	286,080.62	197,501,166.36	This concentration was obtained using a pooled standard curve	290,608
12/6/2020		Bowery Bay	202 6 6 6 6	100 240 224 22	analytical issue	924,695
12/6/2020 12/6/2020		Coney Island Hunts Point	302,640.34 282,330.29		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	682,342 755,948
12/6/2020		Jamaica Bay	662,900.07		this sample was analyzed in duplicate. The higher of the 2 results is	755,948 748,737
12/6/2020		Newtown Creek	264,892.98		This concentration was obtained using a pooled standard curve	1,156,473
12/6/2020		North River	168,722.58		This concentration was obtained using a pooled standard curve	658,596
12/6/2020		Oakwood Beach	362,883.00		This concentration was obtained using a pooled standard curve	258,731
12/6/2020		Owls Head	561,313.98		This concentration was obtained using a pooled standard curve	906,442
12/6/2020		Port Richmond	593,028.02		This concentration was obtained using a pooled standard curve	226,167
12/6/2020			277,192.39		This concentration was obtained using a pooled standard curve	224,029
12/6/2020 12/6/2020		Rockaway Tallman Island	210,693.22 297,643.89		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
12/6/2020		Wards Island	371,673.79		This concentration was obtained using a pooled standard curve	1,201,485
12/8/2020		26th Ward	346,566.03		This concentration was obtained using a pooled standard curve	290,608
12/8/2020		Bowery Bay	485,821.53		This concentration was obtained using a pooled standard curve	924,695
12/8/2020		Coney Island	1,341,883.02		This concentration was obtained using a pooled standard curve	682,342
12/8/2020		Hunts Point	690,020.46		This concentration was obtained using a pooled standard curve	755,948
12/8/2020		Jamaica Bay	747,858.40		This concentration was obtained using a pooled standard curve	748,737
	1 17/9/2020	Newtown Creek	337,431.03	196,599,515.21	this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
12/8/2020 12/8/2020		North River	200,703.09	101 515 001 53	This concentration was obtained using a pooled standard curve	658,596

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1 copies per day per	Annotation	Served,
			(N1 Copies/L)	population)		estimated
12/8/2020	12/9/2020	Owls Head	543,977.59		This concentration was obtained using a pooled standard curve	906,442
12/8/2020	12/9/2020	Port Richmond	918,287.58	399,609,447.98	This concentration was obtained using a pooled standard curve	226,167
12/8/2020			165,236.74	64,215,984.00	This concentration was obtained using a pooled standard curve	224,029
12/8/2020			307,896.19		This concentration was obtained using a pooled standard curve	120,539
12/8/2020		Tallman Island	389,313.87		This concentration was obtained using a pooled standard curve	449,907
12/8/2020	12/9/2020	Wards Island	414,279.87 309,186.81		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
	12/14/2020		386,467.20		This concentration was obtained using a pooled standard curve	924,695
	12/14/2020	, ,	747,322.73		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
12/13/2020	12/14/2020	Hunts Point	348,078.80	212,646,581.97	This concentration was obtained using a pooled standard curve	755,948
	12/14/2020		579,362.08		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	250,755.56		This concentration was obtained using a pooled standard curve	1,156,473
	12/14/2020		231,544.53		This concentration was obtained using a pooled standard curve	658,596
	12/14/2020	Oakwood Beach	1,124,530.35 817,842.07	, ,	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
		Port Richmond	575,045.21		This concentration was obtained using a pooled standard curve	226,167
	12/14/2020		221,322.51		This concentration was obtained using a pooled standard curve	224,029
12/13/2020	12/14/2020	Rockaway	245,773.00	162,083,500.76	This concentration was obtained using a pooled standard curve	120,539
12/13/2020	12/14/2020	Tallman Island	619,906.52	260,787,268.44	This concentration was obtained using a pooled standard curve	449,907
		Wards Island	518,868.13	, ,	This concentration was obtained using a pooled standard curve	1,201,485
	12/16/2020		324,057.03		This concentration was obtained using a pooled standard curve	290,608
	12/16/2020		545,945.87 619,168.41		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
	12/16/2020		397,972.71		This concentration was obtained using a pooled standard curve	755,948
	12/16/2020		660,789.42		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	676,631.56		This concentration was obtained using a pooled standard curve	1,156,473
	12/16/2020		116,795.71		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	653,475.95		This concentration was obtained using a pooled standard curve	258,731
	12/16/2020		734,894.47		This concentration was obtained using a pooled standard curve	906,442
	12/16/2020	Port Richmond	418,419.91 119,571.95		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029
	12/16/2020		333,229.47		this sample was analyzed in duplicate. The higher of the 2 results is	120,539
		Tallman Island	493,432.08		This concentration was obtained using a pooled standard curve	449,907
12/15/2020	12/16/2020	Wards Island	283,433.34	145,557,007.02	This concentration was obtained using a pooled standard curve	1,201,485
12/20/2020	12/21/2020	26th Ward	288,064.37	206,375,243.53	This concentration was obtained using a pooled standard curve	290,608
	12/21/2020		438,074.54		This concentration was obtained using a pooled standard curve	924,695
	12/21/2020		269,019.73		This concentration was obtained using a pooled standard curve	682,342
	12/21/2020		277,142.94 931,694.60		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
		Newtown Creek	187,044.26		This concentration was obtained using a pooled standard curve	1.156.473
	12/21/2020		347,403.56		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	625,333.97		This concentration was obtained using a pooled standard curve	258,731
12/20/2020	12/21/2020	Owls Head	811,158.15	355,686,791.08	This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	630,412.61		This concentration was obtained using a pooled standard curve	226,167
	12/21/2020		128,286.44		This concentration was obtained using a pooled standard curve	224,029
	12/21/2020		132,319.01		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
		Tallman Island Wards Island	471,711.69 380,835.45		this sample was analyzed in duplicate. The higher of the 2 results is	1,201,485
	12/23/2020		131,169.01		This concentration was obtained using a pooled standard curve	290,608
	12/23/2020		531,616.68		This concentration was obtained using a pooled standard curve	924,695
	12/23/2020		443,251.47		This concentration was obtained using a pooled standard curve	682,342
	12/23/2020		413,090.49		This concentration was obtained using a pooled standard curve	755,948
	12/23/2020		912,442.93		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
	12/23/2020	Newtown Creek	302,766.96 210,794.82		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596
		Oakwood Beach	387,893.81		This concentration was obtained using a pooled standard curve	258,731
	12/23/2020		488,619.07		This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	249,401.00		This concentration was obtained using a pooled standard curve	226,167
	12/23/2020		190,695.17		This concentration was obtained using a pooled standard curve	224,029
	12/23/2020		259,624.32		This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	464,724.62		This concentration was obtained using a pooled standard curve	449,907
	12/23/2020	Wards Island	332,709.71 310,129.45		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
	12/28/2020		310,129.45		This concentration was obtained using a pooled standard curve	924,695
	12/28/2020		379,764.33		This concentration was obtained using a pooled standard curve	682,342
	12/28/2020		239,460.48		This concentration was obtained using a pooled standard curve	755,948
	12/28/2020		349,046.69	144,704,155.73	This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	184,945.93		This concentration was obtained using a pooled standard curve	1,156,473
12/27/2020	12/28/2020		262,265.26		This concentration was obtained using a pooled standard curve	658,596
					una concentration was obtained wing a popular standard owner.	1 250 721
12/27/2020	12/28/2020 12/28/2020		474,085.61 1,057,578.80		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
12/27/2020	12/28/2020	Red Hook	256,302.71		this sample was analyzed in duplicate. The higher of the 2 results is	224,029
12/27/2020	12/28/2020	Rockaway	226,043.84	149,072,424.16	This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	313,921.20		This concentration was obtained using a pooled standard curve	449,907
		Wards Island	467,951.53		This concentration was obtained using a pooled standard curve	1,201,485
	12/30/2020		74,635.79		This concentration was obtained using a pooled standard curve	290,608
	12/30/2020	Coney Island	120,325.07 360,871.25		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
	12/30/2020	,	109,056.17		This concentration was obtained using a pooled standard curve	755,948
	12/30/2020		764,552.18		This concentration was obtained using a pooled standard curve	748,737
12/29/2020	12/30/2020	Newtown Creek	84,569.07	46,781,664.66	This concentration was obtained using a pooled standard curve	1,156,473
	12/30/2020		74,008.85		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	90,528.59	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	258,731
	12/30/2020		207,228.26		This concentration was obtained using a pooled standard curve	906,442
	12/30/2020	Port Richmond	24,805.00 340,516.24	, ,	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029
	12/30/2020		320,008.88		This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	516,744.70		This concentration was obtained using a pooled standard curve	449,907
12/29/2020	12/30/2020	Wards Island	539,715.01	243,161,662.66	This concentration was obtained using a pooled standard curve	1,201,485
1/3/2021		26th Ward	418,258.71		This concentration was obtained using a pooled standard curve	290,608
1/3/2021		Bowery Bay	415,300.55		This concentration was obtained using a pooled standard curve	924,695
1/3/2021		Coney Island	474,330.95		This concentration was obtained using a pooled standard curve	682,342
1/3/2021 1/3/2021		Hunts Point Jamaica Bay	391,958.07 579,202.10		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
1/3/2021		Newtown Creek	215,735.13		This concentration was obtained using a pooled standard curve	1,156,473
1/3/2021		North River	229,747.20		This concentration was obtained using a pooled standard curve	658,596
1/3/2021		Oakwood Beach	615,400.64		This concentration was obtained using a pooled standard curve	258,731
1/3/2021	1/4/2021	Owls Head	829,758.55		This concentration was obtained using a pooled standard curve	906,442
1/3/2021		Port Richmond	550,691.25		This concentration was obtained using a pooled standard curve	226,167
1/3/2021		Red Hook	145,813.79		This concentration was obtained using a pooled standard curve	224,029
1/3/2021 1/3/2021		Rockaway Tallman Island	307,882.17 371,578.84		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
1/3/2021		Wards Island	474,246.70		this sample was analyzed in duplicate. The higher of the 2 results is	1,201,485
1/5/2021		26th Ward	565,342.47		This concentration was obtained using a pooled standard curve	290,608
1/5/2021		Bowery Bay	597,696.05		This concentration was obtained using a pooled standard curve	924,695
1/5/2021	1/6/2021	Coney Island	433,999.23	192,614,851.50	This concentration was obtained using a pooled standard curve	682,342
1/5/2021		Hunts Point	616,802.82		This concentration was obtained using a pooled standard curve	755,948
1/5/2021		Jamaica Bay	987,605.05		This concentration was obtained using a pooled standard curve	748,737
1/5/2021 1/5/2021		Newtown Creek North River	437,474.51 356,248.17		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473 658,596
1/5/2021		Oakwood Beach	438,387.64		This concentration was obtained using a pooled standard curve	258,731
1/5/2021		Owls Head	1,270,483.68		This concentration was obtained using a pooled standard curve	906,442
1/5/2021		Port Richmond	529,696.21		This concentration was obtained using a pooled standard curve	226,167
1/5/2021	1/6/2021	Red Hook	243,747.85	102,964,968.14	This concentration was obtained using a pooled standard curve	224,029
1/5/2021		Rockaway	197,516.65		This concentration was obtained using a pooled standard curve	120,539
1/5/2021		Tallman Island	479,756.07		This concentration was obtained using a pooled standard curve	449,907
1/5/2021 1/10/2021		Wards Island	577,973.78		This concentration was obtained using a pooled standard curve	1,201,485
1/10/2021		Bowery Bay	394,705.59 228,818.44		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695
1/10/2021		Coney Island	323,786.81		This concentration was obtained using a pooled standard curve	682,342
1/10/2021		Hunts Point	1,052,546.21		this sample was analyzed in duplicate. The higher of the 2 results is	755,948
1/10/2021		Jamaica Bay	534,304.40		This concentration was obtained using a pooled standard curve	748,737
1/10/2021		Newtown Creek	350,068.99		This concentration was obtained using a pooled standard curve	1,156,473
1/10/2021		North River	228,092.25		This concentration was obtained using a pooled standard curve	658,596
1/10/2021 1/10/2021	1/11/2021	Oakwood Beach	236,712.99		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731
1/10/2021		Port Richmond	644,674.09 230,637.40		This concentration was obtained using a pooled standard curve	906,442 226,167
1/10/2021	1/11/2021		198,508.79		This concentration was obtained using a pooled standard curve	224,029
1/10/2021	1/11/2021		394,153.87		This concentration was obtained using a pooled standard curve	120,539
1/10/2021	1/11/2021	Tallman Island	482,247.47	194,760,721.15	This concentration was obtained using a pooled standard curve	449,907
1/10/2021		Wards Island	424,621.38		This concentration was obtained using a pooled standard curve	1,201,485
1/12/2021	1/13/2021		547,020.23		This concentration was obtained using a pooled standard curve	290,608
1/12/2021 1/12/2021		Bowery Bay Coney Island	680,544.55 702,489.04		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
1/12/2021		Hunts Point	579,246.71		This concentration was obtained using a pooled standard curve	755,948
1/12/2021		Jamaica Bay	882,400.60		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
1/12/2021		Newtown Creek	348,193.24		This concentration was obtained using a pooled standard curve	1,156,473
1/12/2021		North River	288,851.07		This concentration was obtained using a pooled standard curve	658,596
1/12/2021		Oakwood Beach	672,552.61		This concentration was obtained using a pooled standard curve	258,731
1/12/2021		Owls Head	791,244.89		This concentration was obtained using a pooled standard curve	906,442
		Nort Richmond	513,733.70	214 962 030 15	This concentration was obtained using a pooled standard curve	226,167
1/12/2021 1/12/2021	1/13/2021 1/13/2021		369,659.19		This concentration was obtained using a pooled standard curve	224,029

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
1/12/2021	1/13/2021	Tallman Island	577,789.91	population) 247.930.697.73	This concentration was obtained using a pooled standard curve	449,907
1/12/2021		Wards Island	603,259.85		This concentration was obtained using a pooled standard curve	1,201,485
1/17/2021	1/18/2021	26th Ward	246,650.63	163,854,272.67	This concentration was obtained using a pooled standard curve	290,608
1/17/2021	1/18/2021	Bowery Bay	555,421.39	200,087,376.27	This concentration was obtained using a pooled standard curve	924,695
1/17/2021		Coney Island	556,106.80		This concentration was obtained using a pooled standard curve	682,342
1/17/2021		Hunts Point	663,092.99		This concentration was obtained using a pooled standard curve	755,948
1/17/2021 1/17/2021		Jamaica Bay Newtown Creek	529,122.35 279,497.27		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	748,737 1,156,473
1/17/2021		North River	280,185.13	, ,	This concentration was obtained using a pooled standard curve	658,596
1/17/2021		Oakwood Beach	336,331.91		This concentration was obtained using a pooled standard curve	258,731
1/17/2021	1/18/2021	Owls Head	619,534.01	227,677,823.52	This concentration was obtained using a pooled standard curve	906,442
1/17/2021		Port Richmond	477,777.92	215,910,384.89	This concentration was obtained using a pooled standard curve	226,167
1/17/2021	1/18/2021		344,954.97		This concentration was obtained using a pooled standard curve	224,029
1/17/2021	1/18/2021		224,221.68		This concentration was obtained using a pooled standard curve	120,539
1/17/2021 1/17/2021		Tallman Island Wards Island	516,366.96 456,379.57		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907
1/19/2021	1/20/2021		74,200.11		This concentration was obtained using a pooled standard curve	290,608
1, 10, 2021	1, 20, 2021	2011 11414	, ,,200111	10,002,002112	Concentration below Method Limit of Detection;No signal in 1 out of	200,000
1/19/2021	1/20/2021	Bowery Bay			3 RT-qPCR wells, result is obtained by averaging signal from the two	924,695
1/19/2021		Coney Island	151,986.18		This concentration was obtained using a pooled standard curve	682,342
1/19/2021		Hunts Point	215,539.27		This concentration was obtained using a pooled standard curve	755,948
1/19/2021		Jamaica Bay	852,488.65		This concentration was obtained using a pooled standard curve	748,737
1/19/2021		Newtown Creek North River	288,464.58 174,977.97		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596
1/19/2021 1/19/2021		Oakwood Beach	577,582.03	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	258,731
1/19/2021	1/20/2021		535,630.67		This concentration was obtained using a pooled standard curve	906,442
1/19/2021	1/20/2021	Port Richmond	622,950.91		This concentration was obtained using a pooled standard curve	226,167
1/19/2021	1/20/2021	Red Hook			analytical issue	224,029
1/19/2021	1/20/2021		304,468.96		This concentration was obtained using a pooled standard curve	120,539
1/19/2021		Tallman Island	241,176.80		This concentration was obtained using a pooled standard curve	449,907
1/19/2021 1/24/2021	1/20/2021	Wards Island	339,901.76 497,488.15		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485
1/24/2021		Bowery Bay	556,581.46		This concentration was obtained using a pooled standard curve	924,695
1/24/2021		Coney Island	127,646.17		This concentration was obtained using a pooled standard curve	682,342
1/24/2021		Hunts Point	593,950.69		This concentration was obtained using a pooled standard curve	755,948
1/24/2021	1/25/2021	Jamaica Bay	754,097.75	301,188,486.56	this sample was analyzed in duplicate. The higher of the 2 results is	748,737
1/24/2021		Newtown Creek	513,546.71		This concentration was obtained using a pooled standard curve	1,156,473
1/24/2021		North River	485,983.83		This concentration was obtained using a pooled standard curve	658,596
1/24/2021 1/24/2021	1/25/2021	Oakwood Beach	149,566.68 797,747.12		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
1/24/2021		Port Richmond	613,652.90		This concentration was obtained using a pooled standard curve	226,167
1/24/2021	1/25/2021		264,034.21		This concentration was obtained using a pooled standard curve	224,029
1/24/2021	1/25/2021	Rockaway	203,094.82		This concentration was obtained using a pooled standard curve	120,539
1/24/2021		Tallman Island	603,327.20		This concentration was obtained using a pooled standard curve	449,907
1/24/2021		Wards Island	585,279.67		This concentration was obtained using a pooled standard curve	1,201,485
1/26/2021	1/27/2021		819,882.15		this sample was analyzed in duplicate. The higher of the 2 results is	290,608
1/26/2021 1/26/2021		Bowery Bay Coney Island	686,492.68 581,454.10		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
1/26/2021		Hunts Point	442,545.82		This concentration was obtained using a pooled standard curve	755,948
1/26/2021		Jamaica Bay	1,287,423.68		This concentration was obtained using a pooled standard curve	748,737
1/26/2021		Newtown Creek	538,738.72		This concentration was obtained using a pooled standard curve	1,156,473
1/26/2021		North River	672,968.34		This concentration was obtained using a pooled standard curve	658,596
1/26/2021		Oakwood Beach	179,062.49		This concentration was obtained using a pooled standard curve	258,731
1/26/2021	1/27/2021		656,610.79		This concentration was obtained using a pooled standard curve	906,442
1/26/2021 1/26/2021		Port Richmond Red Hook	489,630.50		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029
1/26/2021	1/27/2021 1/27/2021		309,297.51 599,570.50		This concentration was obtained using a pooled standard curve	120,539
1/26/2021		Tallman Island	753,357.26		This concentration was obtained using a pooled standard curve	449,907
1/26/2021		Wards Island	668,938.26		This concentration was obtained using a pooled standard curve	1,201,485
1/31/2021		26th Ward	861,458.53		This concentration was obtained using a pooled standard curve	290,608
1/31/2021		Bowery Bay	786,405.90		This concentration was obtained using a pooled standard curve	924,695
1/31/2021		Coney Island	485,448.66		This concentration was obtained using a pooled standard curve	682,342
1/31/2021 1/31/2021		Hunts Point	684,019.27		This concentration was obtained using a pooled standard curve	755,948
1/31/2021		Jamaica Bay Newtown Creek	1,019,392.29 380,755.25		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737 1,156,473
1/31/2021		North River	779,124.14		This concentration was obtained using a pooled standard curve	658,596
1/31/2021		Oakwood Beach	584,455.02		This concentration was obtained using a pooled standard curve	258,731
1/31/2021		Owls Head	624,201.43	414,471,611.60	This concentration was obtained using a pooled standard curve	906,442
	2/1/2021	Port Richmond	477,579.43	191,840,608.14	This concentration was obtained using a pooled standard curve	226,167
1/31/2021						
1/31/2021 1/31/2021 1/31/2021	2/1/2021	Red Hook Rockaway	460,911.78 375,904.97		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	224,029 120,539

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
1/31/2021	2/1/2021	Wards Island	869,397.19		This concentration was obtained using a pooled standard curve	1,201,485
2/7/2021		26th Ward	466,697.27		This concentration was obtained using a pooled standard curve	290,608
2/7/2021	2/8/2021	Bowery Bay	468,268.50	207,029,921.77	This concentration was obtained using a pooled standard curve	924,695
2/7/2021		Coney Island	558,960.86		This concentration was obtained using a pooled standard curve	682,342
2/7/2021		Hunts Point	378,790.40		This concentration was obtained using a pooled standard curve	755,948
2/7/2021		Jamaica Bay	441,450.56		This concentration was obtained using a pooled standard curve	748,737
2/7/2021 2/7/2021		Newtown Creek North River	270,094.29 155,168.76		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	1,156,473 658,596
2/7/2021		Oakwood Beach	328,675.06	, ,	This concentration was obtained using a pooled standard curve	258,731
2/7/2021		Owls Head	611,281.77		This concentration was obtained using a pooled standard curve	906,442
2/7/2021		Port Richmond	300,776.21		This concentration was obtained using a pooled standard curve	226,167
2/7/2021	2/8/2021	Red Hook	211,198.31	99,921,087.94	This concentration was obtained using a pooled standard curve	224,029
2/7/2021		Rockaway	107,931.50		This concentration was obtained using a pooled standard curve	120,539
2/7/2021		Tallman Island	400,605.61		This concentration was obtained using a pooled standard curve	449,907
2/7/2021	2/8/2021 2/15/2021	Wards Island	467,886.71		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485
2/14/2021 2/14/2021		Bowery Bay	361,548.13 248,672.65		This concentration was obtained using a pooled standard curve	290,608 924,695
2/14/2021		Coney Island	456,389.18		This concentration was obtained using a pooled standard curve	682,342
2/14/2021		Hunts Point	567,426.17	, ,	This concentration was obtained using a pooled standard curve	755,948
2/14/2021		Jamaica Bay	263,327.95		This concentration was obtained using a pooled standard curve	748,737
2/14/2021	2/15/2021	Newtown Creek	304,637.21	176,495,503.22	This concentration was obtained using a pooled standard curve	1,156,473
2/14/2021	2/15/2021	North River	181,836.10	99,288,181.84	This concentration was obtained using a pooled standard curve	658,596
2/14/2021	, ,	Oakwood Beach	87,985.12		This concentration was obtained using a pooled standard curve	258,731
2/14/2021	2/15/2021		667,575.39		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
2/14/2021	, ,	Port Richmond	407,194.42		This concentration was obtained using a pooled standard curve	226,167
2/14/2021	2/15/2021		137,742.53		This concentration was obtained using a pooled standard curve	224,029
2/14/2021 2/14/2021	2/15/2021	Tallman Island	70,220.01 323,235.80		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
2/14/2021		Wards Island	583,655.07		This concentration was obtained using a pooled standard curve	1,201,485
2/21/2021	2/22/2021		299,831.67		This concentration was obtained using a pooled standard curve	290,608
2/21/2021		Bowery Bay	582,355.27		This concentration was obtained using a pooled standard curve	924,695
2/21/2021	2/22/2021	Coney Island	434,508.34	214,535,389.14	This concentration was obtained using a pooled standard curve	682,342
2/21/2021	2/22/2021	Hunts Point	579,050.66	408,843,101.72	This concentration was obtained using a pooled standard curve	755,948
2/21/2021		Jamaica Bay	616,372.09		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
2/21/2021		Newtown Creek	321,370.16		This concentration was obtained using a pooled standard curve	1,156,473
2/21/2021		North River	253,922.57		This concentration was obtained using a pooled standard curve	658,596
2/21/2021 2/21/2021	2/22/2021	Oakwood Beach	247,249.46 770,217.97		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
2/21/2021		Port Richmond	327,144.83		This concentration was obtained using a pooled standard curve	226,167
2/21/2021	2/22/2021		286,480.80		This concentration was obtained using a pooled standard curve	224,029
2/21/2021	2/22/2021		316,607.91		This concentration was obtained using a pooled standard curve	120,539
2/21/2021	2/22/2021	Tallman Island	425,144.37	203,892,601.73	This concentration was obtained using a pooled standard curve	449,907
2/21/2021	2/22/2021	Wards Island	637,615.34	351,553,461.62	This concentration was obtained using a pooled standard curve	1,201,485
2/28/2021		26th Ward	330,151.39	305,335,171.16	This concentration was obtained using a pooled standard curve	290,608
2/28/2021		Bowery Bay	190,519.05		This concentration was obtained using a pooled standard curve	924,695
2/28/2021		Coney Island	429,998.59		This concentration was obtained using a pooled standard curve	682,342
2/28/2021 2/28/2021		Hunts Point Jamaica Bay	161,017.11 470,513.37		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
2/28/2021		Newtown Creek	219,368.73		This concentration was obtained using a pooled standard curve	1,156,473
2/28/2021		North River	429,518.95		this sample was analyzed in duplicate. The higher of the 2 results is	658,596
2/28/2021		Oakwood Beach	170,345.87		This concentration was obtained using a pooled standard curve	258,731
2/28/2021		Owls Head	663,219.80		This concentration was obtained using a pooled standard curve	906,442
2/28/2021	3/1/2021	Port Richmond	169,960.41		This concentration was obtained using a pooled standard curve	226,167
2/28/2021		Red Hook	223,366.32		This concentration was obtained using a pooled standard curve	224,029
2/28/2021		Rockaway	273,870.12		This concentration was obtained using a pooled standard curve	120,539
2/28/2021		Tallman Island	419,968.78		This concentration was obtained using a pooled standard curve	449,907
2/28/2021		Wards Island	360,433.57		This concentration was obtained using a pooled standard curve	1,201,485
3/7/2021 3/7/2021		26th Ward Bowery Bay	185,349.85 409,808.40		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695
3/7/2021		Coney Island	456,396.79		This concentration was obtained using a pooled standard curve	682,342
3/7/2021		Hunts Point	161,526.30		This concentration was obtained using a pooled standard curve	755,948
3/7/2021		Jamaica Bay	483,956.61		This concentration was obtained using a pooled standard curve	748,737
3/7/2021		Newtown Creek	421,113.52		This concentration was obtained using a pooled standard curve	1,156,473
3/7/2021	3/8/2021	North River	243,497.15		This concentration was obtained using a pooled standard curve	658,596
3/7/2021		Oakwood Beach	102,147.45		This concentration was obtained using a pooled standard curve	258,731
3/7/2021		Owls Head	832,715.15		This concentration was obtained using a pooled standard curve	906,442
3/7/2021		Port Richmond	403,414.15		This concentration was obtained using a pooled standard curve	226,167
	3/8/2021	Red Hook	181,009.35		This concentration was obtained using a pooled standard curve	224,029
3/7/2021	2/0/2021	Dockowski				
3/7/2021		Rockaway Taliman Island	220,999.84		This concentration was obtained using a pooled standard curve	120,539
	3/8/2021	Rockaway Tallman Island Wards Island	220,999.84 663,423.80 533,034.86	267,930,690.25	this concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	449,907

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
3/14/2021	3/15/2021	Bowery Bay	244,074.19		This concentration was obtained using a pooled standard curve	924,695
3/14/2021	3/15/2021	Coney Island	464,030.74	180,200,342.57	This concentration was obtained using a pooled standard curve	682,342
3/14/2021		Hunts Point	178,550.12		This concentration was obtained using a pooled standard curve	755,948
3/14/2021		Jamaica Bay	468,525.57		This concentration was obtained using a pooled standard curve	748,737
3/14/2021		Newtown Creek	371,141.83		This concentration was obtained using a pooled standard curve	1,156,473
3/14/2021 3/14/2021		North River Oakwood Beach	153,970.51 189,596.18		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596 258,731
3/14/2021	3/15/2021		689,433.45		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
3/14/2021		Port Richmond	316,173.96	, ,	This concentration was obtained using a pooled standard curve	226,167
3/14/2021	3/15/2021	Red Hook	394,210.28	146,541,054.53	This concentration was obtained using a pooled standard curve	224,029
3/14/2021	3/15/2021		214,265.14		This concentration was obtained using a pooled standard curve	120,539
3/14/2021		Tallman Island	403,014.51		This concentration was obtained using a pooled standard curve	449,907
3/14/2021		Wards Island	284,246.70		This concentration was obtained using a pooled standard curve	1,201,485
3/21/2021 3/21/2021	3/22/2021	Bowery Bay	419,431.82 542,041.37		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	290,608 924,695
3/21/2021		Coney Island	88,178.00		This concentration was obtained using a pooled standard curve	682,342
3/21/2021		Hunts Point	498,222.20		This concentration was obtained using a pooled standard curve	755,948
3/21/2021		Jamaica Bay	529,311.82		This concentration was obtained using a pooled standard curve	748,737
3/21/2021	3/22/2021	Newtown Creek	353,094.11	187,233,225.46	This concentration was obtained using a pooled standard curve	1,156,473
3/21/2021		North River	60,152.65		This concentration was obtained using a pooled standard curve	658,596
3/21/2021		Oakwood Beach	397,255.04		This concentration was obtained using a pooled standard curve	258,731 906,442
3/21/2021 3/21/2021	3/22/2021	Port Richmond	716,732.79 402,124.52		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167
3/21/2021	3/22/2021		264,307.47		This concentration was obtained using a pooled standard curve	220,107
3/21/2021	3/22/2021		43,411.80		This concentration was obtained using a pooled standard curve	120,539
3/21/2021		Tallman Island	267,672.05		This concentration was obtained using a pooled standard curve	449,907
3/21/2021	3/22/2021	Wards Island	228,873.30	115,374,459.64	This concentration was obtained using a pooled standard curve	1,201,485
3/28/2021	3/29/2021		292,743.21		This concentration was obtained using a pooled standard curve	290,608
3/28/2021		Bowery Bay	273,684.31		this sample was analyzed in duplicate. The higher of the 2 results is	924,695
3/28/2021		Coney Island	265,552.52		This concentration was obtained using a pooled standard curve	682,342
3/28/2021 3/28/2021		Hunts Point Jamaica Bay	208,422.54 286,701.74		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
3/28/2021		Newtown Creek	202,989.83		This concentration was obtained using a pooled standard curve	1,156,473
3/28/2021		North River	136,695.01		This concentration was obtained using a pooled standard curve	658,596
3/28/2021	3/29/2021	Oakwood Beach	210,345.44	104,634,879.64	This concentration was obtained using a pooled standard curve	258,731
3/28/2021	3/29/2021		299,479.81		This concentration was obtained using a pooled standard curve	906,442
3/28/2021		Port Richmond	233,154.98		This concentration was obtained using a pooled standard curve	226,167
3/28/2021	3/29/2021		101,164.72		This concentration was obtained using a pooled standard curve	224,029
3/28/2021 3/28/2021	3/29/2021	Tallman Island	267,085.32 345,132.77		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
3/28/2021		Wards Island	336,328.30		This concentration was obtained using a pooled standard curve	1,201,485
4/4/2021		26th Ward	169,477.32		This concentration was obtained using a pooled standard curve	290,608
4/4/2021	4/5/2021	Bowery Bay	450,834.60	164,256,158.24	This concentration was obtained using a pooled standard curve	924,695
4/4/2021	4/5/2021	Coney Island	360,551.47	144,015,947.24	This concentration was obtained using a pooled standard curve	682,342
4/4/2021		Hunts Point	155,539.59		This concentration was obtained using a pooled standard curve	755,948
4/4/2021		Jamaica Bay	290,492.90		This concentration was obtained using a pooled standard curve	748,737
4/4/2021 4/4/2021		Newtown Creek North River	238,563.86 247,067.45		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596
4/4/2021		Oakwood Beach	295,056.54		This concentration was obtained using a pooled standard curve	258,731
4/4/2021		Owls Head	576,801.14		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
4/4/2021		Port Richmond	486,229.18	211,591,419.12	This concentration was obtained using a pooled standard curve	226,167
4/4/2021		Red Hook	155,979.14		This concentration was obtained using a pooled standard curve	224,029
4/4/2021		Rockaway	180,594.67		This concentration was obtained using a pooled standard curve	120,539
4/4/2021		Tallman Island	518,763.33		This concentration was obtained using a pooled standard curve	449,907
4/4/2021 4/11/2021		Wards Island 26th Ward	195,560.83 229,801.16		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
4/11/2021		Bowery Bay	229,801.16		This concentration was obtained using a pooled standard curve	924,695
4/11/2021		Coney Island	198,328.83		This concentration was obtained using a pooled standard curve	682,342
4/11/2021		Hunts Point	157,164.86		This concentration was obtained using a pooled standard curve	755,948
4/11/2021		Jamaica Bay	399,972.15		this sample was analyzed in duplicate. The higher of the 2 results is	748,737
4/11/2021		Newtown Creek	145,545.13		This concentration was obtained using a pooled standard curve	1,156,473
4/11/2021		North River	198,842.11		This concentration was obtained using a pooled standard curve	658,596
4/11/2021		Oakwood Beach	172,109.86		This concentration was obtained using a pooled standard curve	258,731
4/11/2021 4/11/2021		Owls Head Port Richmond	335,378.74 260,509.91		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
4/11/2021	4/12/2021		87,295.47		This concentration was obtained using a pooled standard curve	226,167
4/11/2021	4/12/2021		134,488.08		This concentration was obtained using a pooled standard curve	120,539
4/11/2021		Tallman Island	180,573.14		This concentration was obtained using a pooled standard curve	449,907
4/11/2021		Wards Island	251,734.53		This concentration was obtained using a pooled standard curve	1,201,485
	1/19/2021	26th Ward	133,608.54	83.537.372.82	This concentration was obtained using a pooled standard curve	290,608
4/18/2021						
4/18/2021 4/18/2021 4/18/2021	4/19/2021	Bowery Bay Coney Island	103,370.51 210,177.38	36,815,469.01	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
4/18/2021	1/19/2021	Hunts Point	66,668.70	population)	This concentration was obtained using a pooled standard curve	755,948
4/18/2021		Jamaica Bay	200,627.66		This concentration was obtained using a pooled standard curve	748,737
4/18/2021		Newtown Creek	108,921.38	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
4/18/2021	4/19/2021	North River	101,364.33	52,435,028.35	This concentration was obtained using a pooled standard curve	658,596
4/18/2021	4/19/2021	Oakwood Beach	222,740.33	93,854,653.71	This concentration was obtained using a pooled standard curve	258,731
4/18/2021	4/19/2021		374,542.67		This concentration was obtained using a pooled standard curve	906,442
4/18/2021		Port Richmond	256,215.80		This concentration was obtained using a pooled standard curve	226,167
4/18/2021 4/18/2021	4/19/2021 4/19/2021		101,257.94 70,436.99	, ,	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	224,029 120,539
4/18/2021		Tallman Island	147,772.49		This concentration was obtained using a pooled standard curve	449,907
4/18/2021		Wards Island	201,836.05		This concentration was obtained using a pooled standard curve	1,201,485
4/25/2021	4/26/2021	26th Ward	107,619.35	91,119,028.99	this sample was analyzed in duplicate. The higher of the 2 results is	290,608
4/25/2021	4/26/2021	Bowery Bay	84,719.66		This concentration was obtained using a pooled standard curve	924,695
4/25/2021		Coney Island	161,239.07	, ,	This concentration was obtained using a pooled standard curve	682,342
4/25/2021		Hunts Point	91,131.62		This concentration was obtained using a pooled standard curve	755,948
4/25/2021 4/25/2021		Jamaica Bay Newtown Creek	162,589.16 64,040.69		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737 1,156,473
4/25/2021		North River	64,846.75		This concentration was obtained using a pooled standard curve	658,596
4/25/2021		Oakwood Beach	366,913.39		This concentration was obtained using a pooled standard curve	258,731
4/25/2021		Owls Head	215,243.28		This concentration was obtained using a pooled standard curve	906,442
4/25/2021	4/26/2021	Port Richmond	124,231.50		This concentration was obtained using a pooled standard curve	226,167
4/25/2021	4/26/2021		28,953.80		This concentration was obtained using a pooled standard curve	224,029
4/25/2021	4/26/2021	,	123,226.53		This concentration was obtained using a pooled standard curve	120,539
4/25/2021 4/25/2021		Tallman Island Wards Island	80,696.73 72,953.57	, ,	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907 1,201,485
4/23/2021		26th Ward	285,890.55		This concentration was obtained using a pooled standard curve	290,608
4/27/2021		Bowery Bay	118,707.42		This concentration was obtained using a pooled standard curve	924,695
4/27/2021		Coney Island	169,789.71		This concentration was obtained using a pooled standard curve	682,342
4/27/2021	4/28/2021	Hunts Point	68,118.69	40,932,526.58	This concentration was obtained using a pooled standard curve	755,948
4/27/2021		Jamaica Bay	206,025.95		This concentration was obtained using a pooled standard curve	748,737
4/27/2021		Newtown Creek	82,858.90		This concentration was obtained using a pooled standard curve	1,156,473
4/27/2021 4/27/2021		North River	54,971.04		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596
4/27/2021	4/28/2021	Oakwood Beach Owls Head	355,507.96 501,962.14		This concentration was obtained using a pooled standard curve	258,731 906,442
4/27/2021		Port Richmond	123,334.36		This concentration was obtained using a pooled standard curve	226,167
4/27/2021	4/28/2021		39,035.63		This concentration was obtained using a pooled standard curve	224,029
4/27/2021	4/28/2021	Rockaway	156,624.88	93,454,387.04	this sample was analyzed in duplicate. The higher of the 2 results is	120,539
4/27/2021		Tallman Island	166,408.41		This concentration was obtained using a pooled standard curve	449,907
4/27/2021		Wards Island	139,168.83		This concentration was obtained using a pooled standard curve	1,201,485
5/2/2021 5/2/2021		26th Ward Bowery Bay	56,146.00 131,507.55		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695
5/2/2021		Coney Island	89,397.71		This concentration was obtained using a pooled standard curve	682,342
5/2/2021		Hunts Point	89,175.34		This concentration was obtained using a pooled standard curve	755,948
5/2/2021		Jamaica Bay	70,409.23		This concentration was obtained using a pooled standard curve	748,737
5/2/2021		Newtown Creek	292,320.64		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
5/2/2021		North River	51,533.06		This concentration was obtained using a pooled standard curve	658,596
5/2/2021		Oakwood Beach	246,965.55		This concentration was obtained using a pooled standard curve	258,731
5/2/2021		Owls Head	107,549.29		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442
5/2/2021 5/2/2021		Port Richmond Red Hook	178,015.27 86,611.70		This concentration was obtained using a pooled standard curve	226,167 224,029
5/2/2021		Rockaway	215,093.59		This concentration was obtained using a pooled standard curve	120,539
5/2/2021		Tallman Island	79,550.55		This concentration was obtained using a pooled standard curve	449,907
5/2/2021		Wards Island	157,383.53		This concentration was obtained using a pooled standard curve	1,201,485
5/9/2021		26th Ward	129,046.63		This concentration was obtained using a pooled standard curve	290,608
5/9/2021		Bowery Bay	52,198.18		This concentration was obtained using a pooled standard curve	924,695
5/9/2021 5/9/2021		Coney Island Hunts Point	111,495.39 45,375.61		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	682,342 755,948
5/9/2021		Jamaica Bay	61,335.74		This concentration was obtained using a pooled standard curve	755,948 748,737
5/9/2021		Newtown Creek	43,032.77		This concentration was obtained using a pooled standard curve	1,156,473
5/9/2021		North River	60,564.96		This concentration was obtained using a pooled standard curve	658,596
5/9/2021	5/10/2021	Oakwood Beach	232,508.93		This concentration was obtained using a pooled standard curve	258,731
5/9/2021		Owls Head	113,236.78		This concentration was obtained using a pooled standard curve	906,442
5/9/2021		Port Richmond	68,050.77		This concentration was obtained using a pooled standard curve	226,167
5/9/2021 5/9/2021	5/10/2021		33,876.86 25,994.90		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	224,029 120,539
5/9/2021	5/10/2021 5/10/2021	Tallman Island	25,994.90 54,403.74		This concentration was obtained using a pooled standard curve	449,907
5/9/2021		Wards Island	70,702.46		This concentration was obtained using a pooled standard curve	1,201,485
5/11/2021		26th Ward	59,120.52		This concentration was obtained using a pooled standard curve	290,608
5/11/2021	5/12/2021	Bowery Bay	59,376.32	20,174,639.73	This concentration was obtained using a pooled standard curve	924,695
5/11/2021		Coney Island	89,624.80		This concentration was obtained using a pooled standard curve	682,342
5/11/2021	5/12/2021	Hunts Point	50,154.64 39,456.62		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
5/11/2021	5/12/2021					

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
Sample Date	restuate	with Name	(N1 Copies/L)	copies per day per	Annotation	estimated
				population)		
5/11/2021		Newtown Creek	32,410.06		This concentration was obtained using a pooled standard curve	1,156,473
5/11/2021		North River	33,417.26	, ,	This concentration was obtained using a pooled standard curve	658,596
5/11/2021		Oakwood Beach	126,782.01		this sample was analyzed in duplicate. The higher of the 2 results is	258,731
5/11/2021	5/12/2021		83,895.93		This concentration was obtained using a pooled standard curve	906,442
5/11/2021 5/11/2021	5/12/2021	Port Richmond	41,013.81 43,173.88		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167
5/11/2021	5/12/2021		55,023.02		This concentration was obtained using a pooled standard curve	120,539
5/11/2021		Tallman Island	36,906.44		This concentration was obtained using a pooled standard curve	449,907
5/11/2021		Wards Island	60,642.06	, ,	This concentration was obtained using a pooled standard curve	1,201,485
5/16/2021	5/17/2021		48,289.43		This concentration was obtained using a pooled standard curve	290,608
5/16/2021	5/17/2021	Bowery Bay	36,153.07		This concentration was obtained using a pooled standard curve	924,695
5/16/2021	5/17/2021	Coney Island	62,655.43	25,721,795.98	This concentration was obtained using a pooled standard curve	682,342
5/16/2021	5/17/2021	Hunts Point	32,496.50	17,574,436.41	This concentration was obtained using a pooled standard curve	755,948
5/16/2021	5/17/2021	Jamaica Bay	89,548.84	33,955,086.92	This concentration was obtained using a pooled standard curve	748,737
5/16/2021		Newtown Creek	40,356.12		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
5/16/2021		North River	21,848.96		This concentration was obtained using a pooled standard curve	658,596
5/16/2021		Oakwood Beach	88,862.60		This concentration was obtained using a pooled standard curve	258,731
5/16/2021	5/17/2021		130,924.61		This concentration was obtained using a pooled standard curve	906,442
5/16/2021		Port Richmond	32,625.03		This concentration was obtained using a pooled standard curve	226,167
5/16/2021	5/17/2021		17,276.04		This concentration was obtained using a pooled standard curve	224,029
5/16/2021	5/17/2021		27,277.66		This concentration was obtained using a pooled standard curve	120,539 449,907
5/16/2021 5/16/2021		Tallman Island Wards Island	64,578.18 92,630.95		This concentration was obtained using a pooled standard curve	1,201,485
5/18/2021	5/19/2021		46,494.42		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608
5/18/2021		Bowery Bay	115,358.27	, ,	This concentration was obtained using a pooled standard curve	924,695
5/18/2021		Coney Island	108,590.92		This concentration was obtained using a pooled standard curve	682,342
5/18/2021		Hunts Point	43,051.18		This concentration was obtained using a pooled standard curve	755,948
5/18/2021		Jamaica Bay	87,699.28	, ,	This concentration was obtained using a pooled standard curve	748,737
5/18/2021		Newtown Creek	97,458.21		This concentration was obtained using a pooled standard curve	1,156,473
5/18/2021	5/19/2021	North River	31,543.74	15,954,740.51	This concentration was obtained using a pooled standard curve	658,596
5/18/2021	5/19/2021	Oakwood Beach	151,269.54	54,001,495.05	This concentration was obtained using a pooled standard curve	258,731
5/18/2021	5/19/2021	Owls Head	130,762.60	46,416,820.83	This concentration was obtained using a pooled standard curve	906,442
5/18/2021	5/19/2021	Port Richmond	40,459.94	15,575,310.64	this sample was analyzed in duplicate. The higher of the 2 results is	226,167
5/18/2021	5/19/2021	Red Hook	28,019.72	9,942,412.98	This concentration was obtained using a pooled standard curve	224,029
5/18/2021	5/19/2021	,	9,072.47		Concentration below Method Limit of Quantification (above Method	120,539
5/18/2021		Tallman Island	38,907.94		This concentration was obtained using a pooled standard curve	449,907
5/18/2021		Wards Island	41,205.80		This concentration was obtained using a pooled standard curve	1,201,485
5/23/2021 5/23/2021	5/24/2021	Bowery Bay	209,893.30		This concentration was obtained using a pooled standard curve	290,608
5/23/2021		Coney Island	61,769.90 45,495.49		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695
5/23/2021		Hunts Point	18,927.06		This concentration was obtained using a pooled standard curve	755,948
5/23/2021		Jamaica Bay	12,860.52	, ,	Concentration below Method Limit of Quantification (above Method	748,737
5/23/2021		Newtown Creek	19,559.69		This concentration was obtained using a pooled standard curve	1,156,473
5/23/2021		North River	16,834.21	, ,	This concentration was obtained using a pooled standard curve	658,596
5/23/2021		Oakwood Beach	305,832.13		This concentration was obtained using a pooled standard curve	258,731
5/23/2021		Owls Head	39,888.02		This concentration was obtained using a pooled standard curve	906,442
5/23/2021		Port Richmond	99,368.93		This concentration was obtained using a pooled standard curve	226,167
5/23/2021	5/24/2021	Red Hook	14,597.18	5,179,608.50	Concentration below Method Limit of Quantification (above Method	224,029
5/23/2021	5/24/2021		50,762.32		this sample was analyzed in duplicate. The higher of the 2 results is	120,539
5/23/2021		Tallman Island	16,430.99		This concentration was obtained using a pooled standard curve	449,907
5/23/2021		Wards Island	25,762.19		This concentration was obtained using a pooled standard curve	1,201,485
5/25/2021		26th Ward	24,429.42		This concentration was obtained using a pooled standard curve	290,608
5/25/2021		Bowery Bay	38,202.70		This concentration was obtained using a pooled standard curve	924,695
5/25/2021		Coney Island	25,391.57		This concentration was obtained using a pooled standard curve	682,342
5/25/2021		Hunts Point	33,527.54		This concentration was obtained using a pooled standard curve	755,948
5/25/2021		Jamaica Bay	25,190.59		This concentration was obtained using a pooled standard curve	748,737
5/25/2021		Newtown Creek	31,710.00		This concentration was obtained using a pooled standard curve	1,156,473
5/25/2021 5/25/2021		North River Oakwood Beach	18,664.13		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596
J/ZJ/ZUZI		Oakwood Beach Owls Head	303,268.05 39,473.92		This concentration was obtained using a pooled standard curve	258,731 906,442
			23,608.48		This concentration was obtained using a pooled standard curve	226,167
5/25/2021		Port Richmond		5,555,655.10	This concentration was obtained using a pooled standard curve	220,107
5/25/2021 5/25/2021	5/26/2021	Port Richmond Red Hook		6,411,891 17		
5/25/2021 5/25/2021 5/25/2021	5/26/2021 5/26/2021	Red Hook	17,248.64			120.539
5/25/2021 5/25/2021 5/25/2021 5/25/2021	5/26/2021 5/26/2021 5/26/2021	Red Hook Rockaway	17,248.64 86,290.73	46,067,899.67	This concentration was obtained using a pooled standard curve	120,539 449,907
5/25/2021 5/25/2021 5/25/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021	Red Hook Rockaway Tallman Island	17,248.64 86,290.73 34,363.06	46,067,899.67 14,167,006.65		449,907
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021	Red Hook Rockaway Tallman Island Wards Island	17,248.64 86,290.73	46,067,899.67 14,167,006.65 19,748,964.38	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907 1,201,485
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/31/2021	Red Hook Rockaway Tallman Island Wards Island	17,248.64 86,290.73 34,363.06 38,693.21	46,067,899.67 14,167,006.65 19,748,964.38 7,931,625.46	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	449,907 1,201,485 290,608
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/30/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/31/2021 5/31/2021	Red Hook Rockaway Tallman Island Wards Island 26th Ward	17,248.64 86,290.73 34,363.06 38,693.21 6,150.66	46,067,899.67 14,167,006.65 19,748,964.38 7,931,625.46 4,706,364.51	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is Concentration below Method Limit of Quantification (above Method	
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/30/2021 5/30/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/31/2021 5/31/2021 5/31/2021	Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay	17,248.64 86,290.73 34,363.06 38,693.21 6,150.66 5,273.69	46,067,899.67 14,167,006.65 19,748,964.38 7,931,625.46 4,706,364.51 12,547,054.34	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method	449,907 1,201,485 290,608 924,695 682,342
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/30/2021 5/30/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/31/2021 5/31/2021 5/31/2021	Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island	17,248.64 86,290.73 34,363.06 38,693.21 6,150.66 5,273.69 16,508.61	46,067,899.67 14,167,006.65 19,748,964.38 7,931,625.46 4,706,364.51 12,547,054.34 10,840,180.37 12,930,042.84	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve Concentration below Method Limit of Quantification (above Method this sample was analyzed in duplicate. The higher of the 2 results is	449,907 1,201,485 290,608 924,695
5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/25/2021 5/30/2021 5/30/2021 5/30/2021	5/26/2021 5/26/2021 5/26/2021 5/26/2021 5/31/2021 5/31/2021 5/31/2021 5/31/2021 5/31/2021	Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point	17,248.64 86,290.73 34,363.06 38,693.21 6,150.66 5,273.69 16,508.61 9,095.75	46,067,899.67 14,167,006.65 19,748,964.38 7,931,625.46 4,706,364.51 12,547,054.34 10,840,180.37 12,930,042.84 12,381,026.55	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve Concentration below Method Limit of Quantification (above Method	449,907 1,201,485 290,608 924,695 682,342 755,948

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1 copies per day per	Annotation	Served,
			(N1 Copies/L)	population)		estimated
5/30/2021	5/31/2021	Oakwood Beach	24,897.20	14,898,354.21	This concentration was obtained using a pooled standard curve	258,731
5/30/2021	5/31/2021		34,932.93		This concentration was obtained using a pooled standard curve	906,442
5/30/2021		Port Richmond	6,631.89		Concentration below Method Limit of Quantification (above Method	226,167
5/30/2021 5/30/2021	5/31/2021 5/31/2021		10,612.31 13,326.07		Concentration below Method Limit of Quantification (above Method Concentration below Method Limit of Quantification (above Method	224,029 120,539
5/30/2021		Tallman Island	22,420.75	, ,	This concentration was obtained using a pooled standard curve	449,907
5/30/2021		Wards Island	10,958.16		Concentration below Method Limit of Quantification (above Method	1,201,485
6/1/2021		26th Ward	27,909.35	16,359,409.89	This concentration was obtained using a pooled standard curve	290,608
6/1/2021	6/2/2021	Bowery Bay	18,440.84	6,643,207.80	This concentration was obtained using a pooled standard curve	924,695
6/1/2021		Coney Island	15,605.26		This concentration was obtained using a pooled standard curve	682,342
6/1/2021		Hunts Point	8,676.81	5,083,548.40	Concentration below Method Limit of Quantification (above Method	755,948
6/1/2021 6/1/2021		Jamaica Bay Newtown Creek	7,546.34	1 125 059 62	Concentration below Method Limit of Detection; This concentration Concentration below Method Limit of Quantification (above Method	748,737 1,156,473
6/1/2021		North River	5,982.80		Concentration below Method Limit of Quantification (above Method	658,596
6/1/2021		Oakwood Beach	252,691.35		This concentration was obtained using a pooled standard curve	258,731
6/1/2021		Owls Head	68,061.85		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
6/1/2021	6/2/2021	Port Richmond	5,341.49	2,324,446.63	Concentration below Method Limit of Quantification (above Method	226,167
6/1/2021	6/2/2021		61,976.79		This concentration was obtained using a pooled standard curve	224,029
6/1/2021	6/2/2021		18,683.15	12,321,248.63	This concentration was obtained using a pooled standard curve	120,539
6/1/2021		Tallman Island	20.700.00	20.024 545 65	Concentration below Method Limit of Detection; This concentration	449,907
6/1/2021 6/6/2021		Wards Island 26th Ward	39,769.03 13,994.13		This concentration was obtained using a pooled standard curve Concentration below Method Limit of Quantification (above Method	1,201,485 290,608
6/6/2021		Bowery Bay	15,622.98	, ,	This concentration was obtained using a pooled standard curve	924,695
6/6/2021		Coney Island	21,314.68		This concentration was obtained using a pooled standard curve	682,342
6/6/2021		Hunts Point	16,972.04	11,133,362.05	This concentration was obtained using a pooled standard curve	755,948
6/6/2021	6/7/2021	Jamaica Bay	20,017.30	7,792,553.49	This concentration was obtained using a pooled standard curve	748,737
6/6/2021		Newtown Creek	14,110.38		Concentration below Method Limit of Quantification (above Method	1,156,473
6/6/2021		North River	22,751.86		This concentration was obtained using a pooled standard curve	658,596
6/6/2021 6/6/2021		Oakwood Beach Owls Head	90,849.22 41,129.85		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	258,731 906,442
6/6/2021		Port Richmond	27,797.39		This concentration was obtained using a pooled standard curve	226,167
-, -, -	-, , -		,	-,,	Concentration below Method Limit of Detection; No signal in 1 out of	-, -
6/6/2021	6/7/2021	Red Hook			3 RT-qPCR wells, result is obtained by averaging signal from the two	224,029
6/6/2021	6/7/2021	Rockaway	13,927.99		Concentration below Method Limit of Quantification (above Method	120,539
6/6/2021		Tallman Island	36,773.88		This concentration was obtained using a pooled standard curve	449,907
6/6/2021		Wards Island	29,994.59		No signal in 1 out of 3 RT-qPCR wells, result is obtained by averaging	1,201,485
6/8/2021 6/8/2021		26th Ward Bowery Bay	7,070.17 58,234.25		Concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve	290,608 924,695
6/8/2021		Coney Island	27,623.14		This concentration was obtained using a pooled standard curve	682,342
6/8/2021		Hunts Point	9,388.90		Concentration below Method Limit of Quantification (above Method	755,948
6/8/2021	6/9/2021	Jamaica Bay	10,521.80	4,787,581.44	Concentration below Method Limit of Quantification (above Method	748,737
6/8/2021		Newtown Creek			Concentration below Method Limit of Detection; This concentration	1,156,473
6/8/2021		North River	23,650.57	, ,	This concentration was obtained using a pooled standard curve	658,596
6/8/2021		Oakwood Beach	75,809.00		This concentration was obtained using a pooled standard curve	258,731
6/8/2021 6/8/2021		Owls Head Port Richmond	80,084.56 15,080.86		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
6/8/2021	6/9/2021		15,080.80	15,125,410.50	Concentration below Method Limit of Detection; This concentration	220,107
6/8/2021		Rockaway	11,602.39	7,651,595.07		120,539
6/8/2021		Tallman Island	7,868.12	4,634,036.08		449,907
					Concentration below Method Limit of Quantification (above Method	
6/8/2021		Wards Island	9,042.14		Limit of Detection); this sample was analyzed in duplicate. The	1,201,485
6/13/2021	6/14/2021		14,809.26		Concentration below Method Limit of Quantification (above Method	290,608
6/13/2021 6/13/2021		Bowery Bay	11,218.50 32,047.18		Concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve	924,695 682,342
6/13/2021		Coney Island Hunts Point	23,591.38		This concentration was obtained using a pooled standard curve	755,948
6/13/2021		Jamaica Bay	16,538.45		This concentration was obtained using a pooled standard curve	748,737
			,		Concentration below Method Limit of Quantification (above Method	-,
6/13/2021		Newtown Creek	7,134.41		Limit of Detection); this sample was analyzed in duplicate. The	1,156,473
6/13/2021		North River	40,768.44		This concentration was obtained using a pooled standard curve	658,596
6/13/2021		Oakwood Beach	108,725.90		This concentration was obtained using a pooled standard curve	258,731
6/13/2021	6/14/2021		294,013.03		This concentration was obtained using a pooled standard curve	906,442
6/13/2021 6/13/2021	6/14/2021 6/14/2021	Port Richmond Red Hook	49,714.91 36,665.12		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029
6/13/2021	6/14/2021		21,530.81	14,875,389.34		120,539
6/13/2021		Tallman Island	12,739.02	5,680,702.94		449,907
6/13/2021		Wards Island	32,862.59		This concentration was obtained using a pooled standard curve	1,201,485
6/15/2021	6/16/2021		8,461.03		Concentration below Method Limit of Quantification (above Method	290,608
	6/16/2021	Bowery Bay	24,435.37		This concentration was obtained using a pooled standard curve	924,695
6/15/2021				C 20C 242 70	Concentration below Mathematication (above Mathematication)	682,342
6/15/2021		Coney Island	14,758.59		Concentration below Method Limit of Quantification (above Method	-
	6/16/2021	Coney Island Hunts Point Jamaica Bay	14,758.59 16,274.21 6,630.26	9,371,706.55	This concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve Concentration below Method Limit of Quantification (above Method	755,948

			Concentration SARS-	Per capita SARS- CoV-2 load (N1		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target (N1 Copies/L)	copies per day per	Annotation	Served, estimated
6/15/2021	6/16/2021	North River	24,714.11	12 404 674 48	This concentration was obtained using a pooled standard curve	658,596
6/15/2021		Oakwood Beach	90,062.24		this sample was analyzed in duplicate. The higher of the 2 results is	258,731
6/15/2021	6/16/2021		193,304.59	, ,	This concentration was obtained using a pooled standard curve	906,442
6/15/2021	6/16/2021	Port Richmond	14,329.01		Concentration below Method Limit of Quantification (above Method	226,167
6/15/2021	6/16/2021	Red Hook	15,283.77	6,197,984.85	This concentration was obtained using a pooled standard curve	224,029
6/15/2021	6/16/2021	Rockaway	24,018.98	15,840,149.42	This concentration was obtained using a pooled standard curve	120,539
6/15/2021		Tallman Island	13,484.57		Concentration below Method Limit of Quantification (above Method	449,907
6/15/2021		Wards Island	29,018.49		This concentration was obtained using a pooled standard curve	1,201,485
6/20/2021	6/21/2021		21,033.58		This concentration was obtained using a pooled standard curve	290,608
6/20/2021		Bowery Bay	22,512.91	8,017,987.63	This concentration was obtained using a pooled standard curve	924,695
6/20/2021 6/20/2021		Coney Island Hunts Point	15,816.66	10 454 657 40	Concentration below Method Limit of Detection; This concentration	682,342 755,948
6/20/2021		Jamaica Bay	16,324.85		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	735,948 748,737
6/20/2021		Newtown Creek	10,524.05	0,107,515.40	Concentration below Method Limit of Detection;This concentration	1,156,473
6/20/2021		North River	31,024.94	16,405,616.12	This concentration was obtained using a pooled standard curve	658,596
6/20/2021		Oakwood Beach	73,269.77		This concentration was obtained using a pooled standard curve	258,731
6/20/2021	6/21/2021		197,168.02		This concentration was obtained using a pooled standard curve	906,442
6/20/2021	6/21/2021	Port Richmond	26,078.02	10,911,848.48	This concentration was obtained using a pooled standard curve	226,167
6/20/2021	6/21/2021	Red Hook	36,417.88	12,922,384.01	This concentration was obtained using a pooled standard curve	224,029
6/20/2021	6/21/2021	Rockaway	5,475.90	3,611,271.66	Concentration below Method Limit of Quantification (above Method	120,539
6/20/2021		Tallman Island	6,107.13	, ,	Concentration below Method Limit of Quantification (above Method	449,907
6/20/2021		Wards Island	16,808.48		This concentration was obtained using a pooled standard curve	1,201,485
6/22/2021	6/23/2021		30,808.70		This concentration was obtained using a pooled standard curve	290,608
6/22/2021		Bowery Bay	10,125.23		Concentration below Method Limit of Quantification (above Method	924,695
6/22/2021		Coney Island	107,697.08		This concentration was obtained using a pooled standard curve	682,342
6/22/2021		Hunts Point Jamaica Bay	22,984.45 18,337.05		This concentration was obtained using a pooled standard curve	755,948
6/22/2021 6/22/2021		Newtown Creek	23,321.57		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737 1,156,473
6/22/2021		North River	12,212.76		Concentration below Method Limit of Quantification (above Method	658,596
6/22/2021		Oakwood Beach	73,044.20		This concentration was obtained using a pooled standard curve	258,731
6/22/2021	6/23/2021		189,760.40		This concentration was obtained using a pooled standard curve	906,442
6/22/2021		Port Richmond	27,517.90		This concentration was obtained using a pooled standard curve	226,167
6/22/2021	6/23/2021		7,324.91		Concentration below Method Limit of Quantification (above Method	224,029
6/22/2021	6/23/2021	Rockaway			Concentration below Method Limit of Detection; This concentration	120,539
6/22/2021	6/23/2021	Tallman Island	15,132.66	8,275,966.90	this sample was analyzed in duplicate. The higher of the 2 results is	449,907
6/22/2021	6/23/2021	Wards Island	26,427.42	17,734,910.48	This concentration was obtained using a pooled standard curve	1,201,485
6/27/2021	6/28/2021		16,987.99		This concentration was obtained using a pooled standard curve	290,608
6/27/2021		Bowery Bay	32,342.96		This concentration was obtained using a pooled standard curve	924,695
6/27/2021		Coney Island	18,541.20		This concentration was obtained using a pooled standard curve	682,342
6/27/2021		Hunts Point	10,315.80		Concentration below Method Limit of Quantification (above Method	755,948
6/27/2021 6/27/2021		Jamaica Bay	8,562.24		Concentration below Method Limit of Quantification (above Method	748,737
6/27/2021		Newtown Creek North River	32,195.28 16,392.97		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596
6/27/2021		Oakwood Beach	191,479.77		This concentration was obtained using a pooled standard curve	258,731
6/27/2021		Owls Head	75,304.01		This concentration was obtained using a pooled standard curve	906,442
6/27/2021		Port Richmond	58,569.06		This concentration was obtained using a pooled standard curve	226,167
			,		Concentration below Method Limit of Quantification (above Method	,
6/27/2021	6/28/2021	Red Hook	11,558.69	4,296,748.01	Limit of Detection); this sample was analyzed in duplicate. The	224,029
6/27/2021	6/28/2021		4,884.95	3,374,957.67	Concentration below Method Limit of Quantification (above Method	120,539
6/27/2021	6/28/2021	Tallman Island	12,051.73	5,070,019.43	Concentration below Method Limit of Quantification (above Method	449,907
6/27/2021		Wards Island	60,535.75		This concentration was obtained using a pooled standard curve	1,201,485
6/29/2021	6/30/2021		9,765.35	, ,	Concentration below Method Limit of Quantification (above Method	290,608
6/29/2021		Bowery Bay	23,518.17		This concentration was obtained using a pooled standard curve	924,695
6/29/2021		Coney Island	42,980.88		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
6/29/2021		Hunts Point	18,773.31		This concentration was obtained using a pooled standard curve	755,948
6/29/2021		Jamaica Bay	40,891.22		This concentration was obtained using a pooled standard curve	748,737
6/29/2021		Newtown Creek	26,038.64		This concentration was obtained using a pooled standard curve	1,156,473
6/29/2021 6/29/2021		North River Oakwood Beach	4,803.88 281,153.08		Concentration below Method Limit of Quantification (above Method This concentration was obtained using a pooled standard curve	658,596 258,731
6/29/2021	6/30/2021		146,814.47		This concentration was obtained using a pooled standard curve	906,442
6/29/2021		Port Richmond	48,806.28		This concentration was obtained using a pooled standard curve	226,167
6/29/2021	6/30/2021		13,258.43		Concentration below Method Limit of Quantification (above Method	224,029
6/29/2021	6/30/2021		10,559.70		Concentration below Method Limit of Quantification (above Method	120,539
6/29/2021		Tallman Island	61,920.22		This concentration was obtained using a pooled standard curve	449,907
6/29/2021		Wards Island	32,560.01		This concentration was obtained using a pooled standard curve	1,201,485
7/6/2021		26th Ward	19,312.11		This concentration was obtained using a pooled standard curve	290,608
	7/7/2021	Bowery Bay	49,030.91		This concentration was obtained using a pooled standard curve	924,695
7/6/2021			20 404 00	21 004 024 92	This concentration was obtained using a pooled standard curve	682,342
7/6/2021	7/7/2021		39,484.89			-
7/6/2021 7/6/2021	7/7/2021	Hunts Point	28,023.87	22,593,056.50	This concentration was obtained using a pooled standard curve	755,948
7/6/2021	7/7/2021 7/7/2021			22,593,056.50 9,456,482.03		-

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
7/6/2021	7/7/2021	Oakwood Beach	521,239.97	population) 314 195 083 26	this sample was analyzed in duplicate. The higher of the 2 results is	258,731
7/6/2021		Owls Head	177,381.06		This concentration was obtained using a pooled standard curve	906,442
7/6/2021		Port Richmond	35,533.88	, ,	This concentration was obtained using a pooled standard curve	226,167
7/6/2021	7/7/2021	Red Hook	23,156.45	13,694,581.66	This concentration was obtained using a pooled standard curve	224,029
7/6/2021	7/7/2021		17,065.36		This concentration was obtained using a pooled standard curve	120,539
7/6/2021		Tallman Island	27,461.20		This concentration was obtained using a pooled standard curve	449,907
7/6/2021 7/11/2021	7/12/2021	Wards Island	73,705.13 33,851.67		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485
7/11/2021		Bowery Bay	59,563.92	, ,	This concentration was obtained using a pooled standard curve	924,695
7/11/2021		Coney Island	29,006.16	, ,	This concentration was obtained using a pooled standard curve	682,342
7/11/2021	7/12/2021	Hunts Point	27,661.82	22,993,748.24	this sample was analyzed in duplicate. The higher of the 2 results is	755,948
7/11/2021		Jamaica Bay	71,970.56		This concentration was obtained using a pooled standard curve	748,737
7/11/2021		Newtown Creek	27,635.32		This concentration was obtained using a pooled standard curve	1,156,473
7/11/2021 7/11/2021		North River Oakwood Beach	46,110.22 313,278.71		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596 258,731
7/11/2021	7/12/2021		20,216.16		This concentration was obtained using a pooled standard curve	906,442
7/11/2021		Port Richmond	11,249.16		Concentration below Method Limit of Quantification (above Method	226,167
7/11/2021	7/12/2021	Red Hook	141,783.67	93,432,833.26	This concentration was obtained using a pooled standard curve	224,029
7/11/2021	7/12/2021		11,967.89		Concentration below Method Limit of Quantification (above Method	120,539
7/11/2021		Tallman Island	30,355.69		This concentration was obtained using a pooled standard curve	449,907
7/11/2021 7/13/2021	7/12/2021 7/14/2021	Wards Island	85,705.12 52,571.58		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
7/13/2021		Bowery Bay	86,215.26		This concentration was obtained using a pooled standard curve	924,695
7/13/2021		Coney Island	55,141.50		This concentration was obtained using a pooled standard curve	682,342
7/13/2021		Hunts Point	61,168.00		This concentration was obtained using a pooled standard curve	755,948
7/13/2021	7/14/2021	Jamaica Bay	82,154.59	31,982,041.25	This concentration was obtained using a pooled standard curve	748,737
7/13/2021		Newtown Creek	51,486.84	33,705,724.41	This concentration was obtained using a pooled standard curve	1,156,473
7/13/2021	7/14/2021		20,026,20	14 242 450 00	No signal detected; possible analytical issue	658,596
7/13/2021 7/13/2021	7/14/2021	Oakwood Beach	30,826.39 281,093.52		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
7/13/2021		Port Richmond	44,217.38		This concentration was obtained using a pooled standard curve	226,167
7/13/2021	7/14/2021		95,886.24		this sample was analyzed in duplicate. The higher of the 2 results is	224,029
7/13/2021	7/14/2021	Rockaway	12,247.04	8,076,732.14	Concentration below Method Limit of Quantification (above Method	120,539
7/13/2021	7/14/2021	Tallman Island	44,152.00		This concentration was obtained using a pooled standard curve	449,907
7/13/2021		Wards Island	58,769.37		This concentration was obtained using a pooled standard curve	1,201,485
7/18/2021	7/19/2021		59,557.54		This concentration was obtained using a pooled standard curve	290,608
7/18/2021 7/18/2021		Bowery Bay Coney Island	89,683.94 59,619.37		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695
7/18/2021		Hunts Point	53,408.70		This concentration was obtained using a pooled standard curve	755,948
7/18/2021		Jamaica Bay	65,707.63		This concentration was obtained using a pooled standard curve	748,737
7/18/2021	7/19/2021	Newtown Creek	88,640.92	54,256,687.69	This concentration was obtained using a pooled standard curve	1,156,473
7/18/2021		North River	54,128.57		This concentration was obtained using a pooled standard curve	658,596
7/18/2021		Oakwood Beach	19,358.36		This concentration was obtained using a pooled standard curve	258,731
7/18/2021 7/18/2021	7/19/2021	Port Richmond	155,909.75 88,714.95		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	906,442 226,167
7/18/2021	7/19/2021		33,519.85	, ,	This concentration was obtained using a pooled standard curve	220,107
7/18/2021	7/19/2021		152,931.82		This concentration was obtained using a pooled standard curve	120,539
7/18/2021		Tallman Island	36,496.76	17,810,353.24	This concentration was obtained using a pooled standard curve	449,907
7/18/2021		Wards Island	64,416.98	, ,	This concentration was obtained using a pooled standard curve	1,201,485
7/20/2021	7/21/2021		56,176.75	36,587,433.58	This concentration was obtained using a pooled standard curve	290,608
7/20/2021 7/20/2021		Bowery Bay				924,695 682,342
7/20/2021		Coney Island Hunts Point				755,948
7/20/2021		Jamaica Bay	223,913.59	86,035,498.36	This concentration was obtained using a pooled standard curve	748,737
7/20/2021		Newtown Creek	156,826.14		This concentration was obtained using a pooled standard curve	1,156,473
7/20/2021		North River				658,596
7/20/2021		Oakwood Beach				258,731
7/20/2021		Owls Head				906,442
7/20/2021 7/20/2021	7/21/2021	Port Richmond Red Hook	121,422.32	43 085 0/1 22	No signal in 1 out of 3 RT-qPCR wells, result is obtained by averaging	226,167 224,029
7/20/2021		Rockaway	121,722.32	-3,003,041.22	The signal and out of a fit of entwend, reducts obtained by averaging	120,539
7/20/2021		Tallman Island				449,907
7/20/2021		Wards Island				1,201,485
7/25/2021	7/26/2021		194,029.61		This concentration was obtained using a pooled standard curve	290,608
7/25/2021		Bowery Bay	108,888.86		This concentration was obtained using a pooled standard curve	924,695
7/25/2021 7/25/2021		Coney Island Hunts Point	78,460.42 106,277.83		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	682,342 755,948
7/25/2021		Jamaica Bay	106,277.83		This concentration was obtained using a pooled standard curve	755,948 748,737
7/25/2021		Newtown Creek	256,277.11		This concentration was obtained using a pooled standard curve	1,156,473
7/25/2021	7/26/2021		106,011.96		This concentration was obtained using a pooled standard curve	658,596
7/25/2021		Oakwood Beach	85,029.58		This concentration was obtained using a pooled standard curve	258,731
7/25/2021	7/26/2021	Owls Head	163,095.87	59,256,381.36	This concentration was obtained using a pooled standard curve	906,442

			Concentration SARS-	Per capita SARS- CoV-2 load (N1		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target (N1 Copies/L)	copies per day per population)	Annotation	Served, estimated
7/25/2021	7/26/2021	Port Richmond	63,950.76		This concentration was obtained using a pooled standard curve	226,167
7/25/2021	7/26/2021		37,137.38		This concentration was obtained using a pooled standard curve	224,029
7/25/2021 7/25/2021	7/26/2021		50,830.43		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
7/25/2021		Tallman Island Wards Island	190,902.20 65,255.56		This concentration was obtained using a pooled standard curve	1,201,485
7/27/2021	7/28/2021		03,235.30	35,005,307.40	analytical issue	290,608
7/27/2021		Bowery Bay			analytical issue	924,695
7/27/2021	7/28/2021	Coney Island			analytical issue	682,342
7/27/2021		Hunts Point			analytical issue	755,948
7/27/2021		Jamaica Bay			analytical issue	748,737
7/27/2021 7/27/2021		Newtown Creek North River	165,940.89	108,632,768.96	original RT-qPCR (7/28/2021) failed, RT-qPCR repeated analytical issue	1,156,473 658,596
7/27/2021		Oakwood Beach	116,447.91	49 578 017 30	original RT-qPCR (7/28/2021) failed, RT-qPCR repeated; This	258,731
7/27/2021	7/28/2021		110,11101	15,576,627,66	analytical issue	906,442
7/27/2021		Port Richmond			analytical issue	226,167
7/27/2021	7/28/2021	Red Hook			analytical issue	224,029
7/27/2021	7/28/2021				analytical issue	120,539
7/27/2021		Tallman Island	C0 070 45	10 007 000 01	analytical issue	449,907
7/27/2021		Wards Island	69,379.45 132,799.56		original RT-qPCR (7/28/2021) failed, RT-qPCR repeated; This This concentration was obtained using a pooled standard curve	1,201,485 290,608
8/1/2021 8/1/2021		26th Ward Bowery Bay	277,579.55		This concentration was obtained using a pooled standard curve	924,695
8/1/2021		Coney Island	145,002.19		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
8/1/2021		Hunts Point	101,369.95		This concentration was obtained using a pooled standard curve	755,948
8/1/2021	8/2/2021	Jamaica Bay	572,926.39	220,138,522.49	This concentration was obtained using a pooled standard curve	748,737
8/1/2021		Newtown Creek	183,239.00		This concentration was obtained using a pooled standard curve	1,156,473
8/1/2021		North River	109,683.05		This concentration was obtained using a pooled standard curve	658,596
8/1/2021 8/1/2021		Oakwood Beach	243,014.13 227,589.65		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
8/1/2021		Owls Head Port Richmond	189,140.10		This concentration was obtained using a pooled standard curve	226,167
8/1/2021		Red Hook	124,288.43		This concentration was obtained using a pooled standard curve	224,029
8/1/2021		Rockaway	43,522.50		This concentration was obtained using a pooled standard curve	120,539
8/1/2021	8/2/2021	Tallman Island	125,886.69	58,254,928.96	This concentration was obtained using a pooled standard curve	449,907
8/1/2021		Wards Island	111,151.42		This concentration was obtained using a pooled standard curve	1,201,485
8/3/2021		26th Ward	213,878.41		This concentration was obtained using a pooled standard curve	290,608
8/3/2021 8/3/2021		Bowery Bay Coney Island	196,098.59		This concentration was obtained using a pooled standard curve	924,695 682,342
8/3/2021		Hunts Point	184,403.97 181,089.44	76,725,963.98	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948
8/3/2021		Jamaica Bay	338,331.88		This concentration was obtained using a pooled standard curve	748,737
8/3/2021		Newtown Creek	219,322.67		This concentration was obtained using a pooled standard curve	1,156,473
8/3/2021	8/4/2021	North River	68,070.63	38,342,434.74	This concentration was obtained using a pooled standard curve	658,596
8/3/2021		Oakwood Beach	161,582.69		This concentration was obtained using a pooled standard curve	258,731
8/3/2021		Owls Head	614,765.56		This concentration was obtained using a pooled standard curve	906,442
8/3/2021		Port Richmond	225,373.58		This concentration was obtained using a pooled standard curve	226,167
8/3/2021 8/3/2021		Red Hook Rockaway	159,783.65 282,909.38		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	224,029 120,539
8/3/2021		Tallman Island	118,477.77	, ,	This concentration was obtained using a pooled standard curve	449,907
8/3/2021		Wards Island	196,801.65		This concentration was obtained using a pooled standard curve	1,201,485
8/8/2021	8/9/2021	26th Ward	265,977.47	228,662,081.39	This concentration was obtained using a pooled standard curve	290,608
8/8/2021		Bowery Bay	319,002.15		This concentration was obtained using a pooled standard curve	924,695
8/8/2021		Coney Island	389,390.75		This concentration was obtained using a pooled standard curve	682,342
8/8/2021 8/8/2021		Hunts Point Jamaica Bay	259,906.15		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
8/8/2021		Newtown Creek	279,034.61 233,251.00		This concentration was obtained using a pooled standard curve	1,156,473
8/8/2021		North River	480,673.38		This concentration was obtained using a pooled standard curve	658,596
8/8/2021		Oakwood Beach	364,015.12		This concentration was obtained using a pooled standard curve	258,731
8/8/2021	8/9/2021	Owls Head	1,314,093.91	603,659,436.04	This concentration was obtained using a pooled standard curve	906,442
8/8/2021	8/9/2021	Port Richmond	256,500.96	120,207,264.65	This concentration was obtained using a pooled standard curve	226,167
8/8/2021		Red Hook	364,257.52		This concentration was obtained using a pooled standard curve	224,029
8/8/2021		Rockaway	98,401.72		This concentration was obtained using a pooled standard curve	120,539
8/8/2021 8/8/2021		Tallman Island Wards Island	165,834.87 298,814.79	101,856,530.16	this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	449,907 1,201,485
8/8/2021 8/10/2021	8/9/2021 8/11/2021		438,234.51	450,961,038.24		290,608
8/10/2021		Bowery Bay	466,181.02	251,908,564.35		924,695
8/10/2021		Coney Island	512,046.83		This concentration was obtained using a pooled standard curve	682,342
8/10/2021		Hunts Point	671,934.54		this sample was analyzed in duplicate. The higher of the 2 results is	755,948
8/10/2021		Jamaica Bay	811,897.04		This concentration was obtained using a pooled standard curve	748,737
8/10/2021		Newtown Creek	197,826.27	163,825,650.16		1,156,473
8/10/2021 8/10/2021		North River Oakwood Beach	188,105.56 415,335.97	148,120,798.36	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596 258,731
8/10/2021	8/11/2021 8/11/2021		670,174.32	316,256,262.21		906,442
8/10/2021		Port Richmond	547,027.06		This concentration was obtained using a pooled standard curve	226,167
			2 ,0200	69,862,565.37		

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
8/10/2021	8/11/2021	Rockaway	240,033.95		This concentration was obtained using a pooled standard curve	120,539
8/10/2021		Tallman Island	284,108.33		This concentration was obtained using a pooled standard curve	449,907
8/10/2021		Wards Island	315,827.44		This concentration was obtained using a pooled standard curve	1,201,485
8/15/2021	8/16/2021	26th Ward	191,884.95	129,971,944.91	This concentration was obtained using a pooled standard curve	290,608
8/15/2021	8/16/2021	Bowery Bay	423,720.84	152,643,007.55	This concentration was obtained using a pooled standard curve	924,695
8/15/2021		Coney Island	272,993.17		This concentration was obtained using a pooled standard curve	682,342
8/15/2021		Hunts Point	136,479.20		This concentration was obtained using a pooled standard curve	755,948
8/15/2021		Jamaica Bay	320,904.49		This concentration was obtained using a pooled standard curve	748,737
8/15/2021		Newtown Creek	138,407.66		This concentration was obtained using a pooled standard curve	1,156,473
8/15/2021		North River	165,486.37		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	658,596
8/15/2021 8/15/2021		Oakwood Beach Owls Head	166,297.93 229,982.80	64,475,865.87	This concentration was obtained using a pooled standard curve	258,731 906,442
8/15/2021		Port Richmond	135,932.68		This concentration was obtained using a pooled standard curve	226,167
8/15/2021	8/16/2021		164,975.97		This concentration was obtained using a pooled standard curve	224,029
8/15/2021	8/16/2021		87,982.41		This concentration was obtained using a pooled standard curve	120,539
8/15/2021		, Tallman Island	97,847.09		This concentration was obtained using a pooled standard curve	449,907
8/15/2021	8/16/2021	Wards Island	108,074.89		This concentration was obtained using a pooled standard curve	1,201,485
8/17/2021	8/18/2021	26th Ward	290,029.81	177,560,291.53	This concentration was obtained using a pooled standard curve	290,608
8/17/2021		Bowery Bay				924,695
8/17/2021	8/18/2021	Coney Island	135,855.52	52,757,736.71	This concentration was obtained using a pooled standard curve	682,342
8/17/2021		Hunts Point				755,948
8/17/2021		Jamaica Bay	343,478.82		This concentration was obtained using a pooled standard curve	748,737
8/17/2021	8/18/2021	Newtown Creek	196,408.14	115,720,261.60	this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
8/17/2021	0/10/2021	North River Oakwood Beach	70 550 70	20 200 200 200	This concentration was obtained using a nearly distant and suc	658,596
8/17/2021			79,556.76		This concentration was obtained using a pooled standard curve	258,731
8/17/2021 8/17/2021	8/18/2021	Port Richmond	224,946.56 190,403.20		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
8/17/2021	8/18/2021		79,476.69		This concentration was obtained using a pooled standard curve	220,107
8/17/2021	8/18/2021		128,165.74		This concentration was obtained using a pooled standard curve	120,539
8/17/2021	0,10,2021	Tallman Island	120,100171	00,100,100,1		449,907
8/17/2021		Wards Island				1,201,485
8/22/2021	8/23/2021	26th Ward	183,155.02	298,218,242.64	This concentration was obtained using a pooled standard curve	290,608
8/22/2021	8/23/2021	Bowery Bay	30,713.30	31,055,447.08	This concentration was obtained using a pooled standard curve	924,695
					Concentration below Method Limit of Quantification (above Method	
8/22/2021	8/23/2021	Coney Island	12,520.51	13,614,097.66	Limit of Detection); this sample was analyzed in duplicate. The	682,342
8/22/2021	8/23/2021	Hunts Point	35,461.10	58,776,158.72	This concentration was obtained using a pooled standard curve	755,948
8/22/2021		Jamaica Bay	202,767.71		This concentration was obtained using a pooled standard curve	748,737
8/22/2021		Newtown Creek	27,532.29		This concentration was obtained using a pooled standard curve	1,156,473
8/22/2021		North River	38,523.22		This concentration was obtained using a pooled standard curve	658,596
8/22/2021		Oakwood Beach	52,764.07		This concentration was obtained using a pooled standard curve	258,731
8/22/2021		Owls Head	58,179.93		This concentration was obtained using a pooled standard curve	906,442
8/22/2021 8/22/2021	8/23/2021	Port Richmond	22,619.96 17,293.46		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	220,107
8/22/2021	8/23/2021		10,442.00		Concentration below Method Limit of Quantification (above Method	120,539
8/22/2021		Tallman Island	38,162.64		This concentration was obtained using a pooled standard curve	449,907
8/22/2021		Wards Island	48,268.66		This concentration was obtained using a pooled standard curve	1,201,485
8/24/2021	8/25/2021		193,977.62		This concentration was obtained using a pooled standard curve	290,608
8/24/2021		Bowery Bay	138,095.70		this sample was analyzed in duplicate. The higher of the 2 results is	924,695
8/24/2021		Coney Island	253,857.40		This concentration was obtained using a pooled standard curve	682,342
8/24/2021	8/25/2021	Hunts Point	202,100.68		This concentration was obtained using a pooled standard curve	755,948
8/24/2021		Jamaica Bay	299,348.82		This concentration was obtained using a pooled standard curve	748,737
8/24/2021		Newtown Creek	160,335.80		This concentration was obtained using a pooled standard curve	1,156,473
8/24/2021		North River	76,174.44		This concentration was obtained using a pooled standard curve	658,596
8/24/2021		Oakwood Beach	41,444.95		This concentration was obtained using a pooled standard curve	258,731
8/24/2021		Owls Head	220,640.34		This concentration was obtained using a pooled standard curve	906,442
8/24/2021		Port Richmond	194,388.12		This concentration was obtained using a pooled standard curve	226,167
8/24/2021 8/24/2021	8/25/2021		98,378.44	51,531,208.32		224,029
8/24/2021 8/24/2021	8/25/2021	Rockaway Tallman Island	370,193.13 64,417.02	325,515,538.15 41,191,198.18	•	120,539 449,907
8/24/2021		Wards Island	197,157.04	139,140,925.48		1,201,485
-, - , 2021	-, -0, 2021		107,107.04		Concentration below Method Limit of Detection;No signal in 1 out of	_,_01,-03
8/29/2021	8/30/2021	26th Ward			3 RT-qPCR wells, result is obtained by averaging signal from the two	290,608
8/29/2021		Bowery Bay			Concentration below Method Limit of Detection; This concentration	924,695
8/29/2021		Coney Island	56,289.41	26,855,669.11	This concentration was obtained using a pooled standard curve	682,342
	·				Concentration below Method Limit of Detection; No signal in 1 out of	
8/29/2021	8/30/2021	Hunts Point			3 RT-qPCR wells, result is obtained by averaging signal from the two	755,948
8/29/2021	8/30/2021	Jamaica Bay	193,572.52	79,270,639.25		748,737
8/29/2021	8/30/2021	Newtown Creek	63,054.12	37,356,781.53	This concentration was obtained using a pooled standard curve	1,156,473
8/29/2021		North River	72,253.68	48,173,902.01		658,596
8/29/2021		Oakwood Beach	94,398.76	48,753,463.48		258,731
8/29/2021 8/29/2021		Owls Head	12,563.56		Concentration below Method Limit of Quantification (above Method	906,442
	8/30/2021	Port Richmond	48,709.97	25,273,376.80	This concentration was obtained using a pooled standard curve	226,167

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
0 /20 /2024	0/20/2024	<u> </u>		population)		
8/29/2021	8/30/2021		29,987.90		This concentration was obtained using a pooled standard curve	224,029
8/29/2021 8/29/2021	8/30/2021	Tallman Island	10,445.24 102,454.67		Concentration below Method Limit of Quantification (above Method this sample was analyzed in duplicate. The higher of the 2 results is	120,539 449,907
8/29/2021		Wards Island	146,918.66		This concentration was obtained using a pooled standard curve	1,201,485
8/31/2021		26th Ward	87,569.27		This concentration was obtained using a pooled standard curve	290,608
8/31/2021		Bowery Bay	122,685.36		This concentration was obtained using a pooled standard curve	924,695
8/31/2021		Coney Island	112,110.88		This concentration was obtained using a pooled standard curve	682,342
8/31/2021		Hunts Point	71,199.67		this sample was analyzed in duplicate. The higher of the 2 results is	755,948
8/31/2021		Jamaica Bay	203,267.83	, ,	This concentration was obtained using a pooled standard curve	748,737
8/31/2021	9/1/2021	Newtown Creek	58,630.87	36,079,594.14	This concentration was obtained using a pooled standard curve	1,156,473
8/31/2021	9/1/2021	North River	45,440.91	31,602,847.71	This concentration was obtained using a pooled standard curve	658,596
8/31/2021	9/1/2021	Oakwood Beach	179,653.77	79,905,078.47	This concentration was obtained using a pooled standard curve	258,731
8/31/2021		Owls Head	194,335.11		This concentration was obtained using a pooled standard curve	906,442
8/31/2021		Port Richmond	46,313.04		This concentration was obtained using a pooled standard curve	226,167
8/31/2021		Red Hook	61,681.49		This concentration was obtained using a pooled standard curve	224,029
8/31/2021		Rockaway	19,546.54		This concentration was obtained using a pooled standard curve	120,539
8/31/2021		Tallman Island	60,400.94		This concentration was obtained using a pooled standard curve	449,907
8/31/2021		Wards Island	51,100.55		This concentration was obtained using a pooled standard curve	1,201,485
9/7/2021		26th Ward	124,548.09		This concentration was obtained using a pooled standard curve	290,608
9/7/2021 9/7/2021		Bowery Bay Coney Island	95,639.54 234,194.25		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695
9/7/2021		Hunts Point	112,578.06		This concentration was obtained using a pooled standard curve	755,948
9/7/2021		Jamaica Bay	133,135.16		This concentration was obtained using a pooled standard curve	748,737
9/7/2021		Newtown Creek	62,796.13		This concentration was obtained using a pooled standard curve	1,156,473
9/7/2021		North River	90,687.84	, ,	this sample was analyzed in duplicate. The higher of the 2 results is	658,596
9/7/2021	9/8/2021	Oakwood Beach	113,901.50	63,158,687.68	This concentration was obtained using a pooled standard curve	258,731
9/7/2021	9/8/2021	Owls Head	110,760.06	39,316,515.18	This concentration was obtained using a pooled standard curve	906,442
9/7/2021	9/8/2021	Port Richmond	116,004.16	66,014,020.97	This concentration was obtained using a pooled standard curve	226,167
9/7/2021	9/8/2021	Red Hook	133,767.09	56,506,444.42	This concentration was obtained using a pooled standard curve	224,029
9/7/2021		Rockaway	85,404.35		This concentration was obtained using a pooled standard curve	120,539
9/7/2021		Tallman Island	36,938.08		This concentration was obtained using a pooled standard curve	449,907
9/7/2021		Wards Island	143,336.57		This concentration was obtained using a pooled standard curve	1,201,485
9/12/2021	9/13/2021		39,278.36		This concentration was obtained using a pooled standard curve	290,608
9/12/2021		Bowery Bay	102,944.58		This concentration was obtained using a pooled standard curve	924,695
9/12/2021 9/12/2021		Coney Island Hunts Point	112,061.18		this sample was analyzed in duplicate. The higher of the 2 results is	682,342 755,948
9/12/2021		Jamaica Bay	128,921.93 141,644.36		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737
9/12/2021		Newtown Creek	186,856.78		This concentration was obtained using a pooled standard curve	1,156,473
9/12/2021		North River	183,247.24		This concentration was obtained using a pooled standard curve	658,596
9/12/2021		Oakwood Beach	338,536.36		This concentration was obtained using a pooled standard curve	258,731
9/12/2021	9/13/2021		328,111.82		This concentration was obtained using a pooled standard curve	906,442
9/12/2021		Port Richmond	154,409.79		This concentration was obtained using a pooled standard curve	226,167
9/12/2021	9/13/2021	Red Hook	106,575.82	54,024,235.83	This concentration was obtained using a pooled standard curve	224,029
9/12/2021	9/13/2021	Rockaway	143,365.26	108,053,917.90	This concentration was obtained using a pooled standard curve	120,539
9/12/2021	9/13/2021	Tallman Island	195,775.21	88,949,115.92	This concentration was obtained using a pooled standard curve	449,907
9/12/2021	9/13/2021	Wards Island	219,156.66	127,738,255.04	This concentration was obtained using a pooled standard curve	1,201,485
9/14/2021	9/15/2021		52,909.07		This concentration was obtained using a pooled standard curve	290,608
9/14/2021		Bowery Bay	79,991.53		This concentration was obtained using a pooled standard curve	924,695
9/14/2021		Coney Island	12,844.28		Concentration below Method Limit of Quantification (above Method	682,342
9/14/2021		Hunts Point	183,130.42		This concentration was obtained using a pooled standard curve	755,948
9/14/2021		Jamaica Bay	474,951.61		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737
9/14/2021 9/14/2021		Newtown Creek North River	111,279.81 126,256.54		This concentration was obtained using a pooled standard curve	1,156,473 658,596
9/14/2021		Oakwood Beach	99,342.09		This concentration was obtained using a pooled standard curve	258,731
9/14/2021	9/15/2021		462,580.36		This concentration was obtained using a pooled standard curve	906,442
9/14/2021		Port Richmond	188,827.03		This concentration was obtained using a pooled standard curve	226,167
9/14/2021	9/15/2021		195,535.70		This concentration was obtained using a pooled standard curve	224,029
9/14/2021	9/15/2021		38,388.94		This concentration was obtained using a pooled standard curve	120,539
9/14/2021		Tallman Island	86,319.42		This concentration was obtained using a pooled standard curve	449,907
9/14/2021		Wards Island	142,560.17		this sample was analyzed in duplicate. The higher of the 2 results is	1,201,485
9/19/2021	9/20/2021	26th Ward	108,236.48	71,903,361.19	This concentration was obtained using a pooled standard curve	290,608
9/19/2021		Bowery Bay	147,056.36		This concentration was obtained using a pooled standard curve	924,695
9/19/2021		Coney Island	105,341.59		This concentration was obtained using a pooled standard curve	682,342
9/19/2021		Hunts Point	103,743.19		this sample was analyzed in duplicate. The higher of the 2 results is	755,948
9/19/2021		Jamaica Bay	159,407.35		This concentration was obtained using a pooled standard curve	748,737
9/19/2021		Newtown Creek	138,346.55		This concentration was obtained using a pooled standard curve	1,156,473
9/19/2021		North River	67,355.31		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	484,880.05	217,080,192.52	This concentration was obtained using a pooled standard curve	258,731
9/19/2021			450.053.07	E7 337 037 C0	This concentration was obtained using a part of standard and	000 440
9/19/2021 9/19/2021	9/20/2021	Owls Head	150,852.07		This concentration was obtained using a pooled standard curve	906,442
9/19/2021	9/20/2021	Owls Head Port Richmond	150,852.07 102,756.54 135,246.57	46,436,226.13	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167 224,029

Sample Date 9/19/2021 9/19/2021	Test date	WRRF Name	Concentration SARS- CoV-2 gene target	CoV-2 load (N1	Annotation	Population Served,
						Jerveu,
			(N1 Copies/L)	copies per day per		estimated
	9/20/2021	Tallman Island	81,625.78	population) 35 712 549 52	This concentration was obtained using a pooled standard curve	449,907
		Wards Island	105,243.31		This concentration was obtained using a pooled standard curve	1,201,485
9/21/2021	9/22/2021		188,535.17	, ,	This concentration was obtained using a pooled standard curve	290,608
9/21/2021	9/22/2021	Bowery Bay	106,759.11	41,518,624.94	No signal in 1 out of 3 RT-qPCR wells, result is obtained by averaging	924,695
9/21/2021		Coney Island	149,165.95		This concentration was obtained using a pooled standard curve	682,342
9/21/2021		Hunts Point	88,048.71		This concentration was obtained using a pooled standard curve	755,948
9/21/2021		Jamaica Bay	152,901.06 177,425.59	, ,	This concentration was obtained using a pooled standard curve	748,737 1,156,473
9/21/2021 9/21/2021		Newtown Creek North River	117,124.92		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596
9/21/2021		Oakwood Beach	184,621.04		This concentration was obtained using a pooled standard curve	258,731
9/21/2021	9/22/2021		284,322.52		This concentration was obtained using a pooled standard curve	906,442
9/21/2021	9/22/2021	Port Richmond	97,217.69	42,306,036.23	This concentration was obtained using a pooled standard curve	226,167
9/21/2021	9/22/2021		136,924.38		This concentration was obtained using a pooled standard curve	224,029
9/21/2021	9/22/2021		12,721.37	, ,	Concentration below Method Limit of Quantification (above Method	120,539
9/21/2021		Tallman Island	94,202.41		This concentration was obtained using a pooled standard curve	449,907
9/21/2021 9/26/2021	9/22/2021 9/27/2021	Wards Island	138,084.14 84,601.66		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
9/26/2021		Bowery Bay	49,344.20		This concentration was obtained using a pooled standard curve	924,695
9/26/2021		Coney Island	91,046.73	, ,	This concentration was obtained using a pooled standard curve	682,342
9/26/2021		Hunts Point	66,511.61		This concentration was obtained using a pooled standard curve	755,948
9/26/2021		Jamaica Bay	104,383.59	, ,	This concentration was obtained using a pooled standard curve	748,737
9/26/2021		Newtown Creek	122,249.68		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
9/26/2021		North River	29,858.16 333,441.83		This concentration was obtained using a pooled standard curve	658,596
9/26/2021 9/26/2021	9/27/2021	Oakwood Beach	333,441.83		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
9/26/2021		Port Richmond	61,959.19		This concentration was obtained using a pooled standard curve	226,167
9/26/2021	9/27/2021		108,136.76		This concentration was obtained using a pooled standard curve	224,029
9/26/2021	9/27/2021	Rockaway	155,912.78	102,822,073.00	This concentration was obtained using a pooled standard curve	120,539
9/26/2021	9/27/2021	Tallman Island	79,939.67	45,063,747.17	This concentration was obtained using a pooled standard curve	449,907
9/26/2021		Wards Island	31,183.69		This concentration was obtained using a pooled standard curve	1,201,485
9/28/2021	9/29/2021		97,629.91		This concentration was obtained using a pooled standard curve	290,608
9/28/2021 9/28/2021		Bowery Bay Coney Island	24,928.85 106,985.57		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
9/28/2021		Hunts Point	115,035.60		This concentration was obtained using a pooled standard curve	755,948
9/28/2021		Jamaica Bay	179,073.08		This concentration was obtained using a pooled standard curve	748,737
9/28/2021	9/29/2021	Newtown Creek	121,936.51	78,628,033.92	This concentration was obtained using a pooled standard curve	1,156,473
9/28/2021		North River	104,271.69		this sample was analyzed in duplicate. The higher of the 2 results is	658,596
9/28/2021		Oakwood Beach	261,111.70		This concentration was obtained using a pooled standard curve	258,731
9/28/2021	9/29/2021		168,066.42		This concentration was obtained using a pooled standard curve	906,442
9/28/2021 9/28/2021	9/29/2021 9/29/2021	Port Richmond	125,444.51 238,265.30		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029
9/28/2021	9/29/2021		89,215.90		This concentration was obtained using a pooled standard curve	120,539
9/28/2021	, ,	Tallman Island	90,733.06		This concentration was obtained using a pooled standard curve	449,907
9/28/2021	9/29/2021	Wards Island	87,364.28	53,673,893.05	This concentration was obtained using a pooled standard curve	1,201,485
10/3/2021	10/4/2021		88,625.40	56,566,542.29	This concentration was obtained using a pooled standard curve	290,608
10/3/2021		Bowery Bay	537,208.77		This concentration was obtained using a pooled standard curve	924,695
10/3/2021		Coney Island	216,058.59		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
10/3/2021		Hunts Point	52,693.51		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948
10/3/2021 10/3/2021		Jamaica Bay Newtown Creek	92,628.90 83,817.17		This concentration was obtained using a pooled standard curve	748,737 1,156,473
10/3/2021		North River	88,461.32		This concentration was obtained using a pooled standard curve	658,596
10/3/2021		Oakwood Beach	253,055.09		This concentration was obtained using a pooled standard curve	258,731
10/3/2021	10/4/2021		145,989.09		This concentration was obtained using a pooled standard curve	906,442
10/3/2021		Port Richmond	109,554.48		This concentration was obtained using a pooled standard curve	226,167
10/3/2021	10/4/2021		137,416.36		This concentration was obtained using a pooled standard curve	224,029
10/3/2021 10/3/2021	10/4/2021	Rockaway Tallman Island	41,056.43 94,034.79		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
10/3/2021		Wards Island	94,034.79		This concentration was obtained using a pooled standard curve	449,907
10/5/2021	10/4/2021		147,136.88		This concentration was obtained using a pooled standard curve	290,608
10/5/2021		Bowery Bay	210,210.53		This concentration was obtained using a pooled standard curve	924,695
10/5/2021		Coney Island	123,095.17	51,216,875.73	This concentration was obtained using a pooled standard curve	682,342
10/5/2021		Hunts Point	85,980.68		This concentration was obtained using a pooled standard curve	755,948
10/5/2021		Jamaica Bay	181,469.38		This concentration was obtained using a pooled standard curve	748,737
10/5/2021		Newtown Creek	122,260.69		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473
10/5/2021 10/5/2021		North River Oakwood Beach	386,142.46 219,277.80		this sample was analyzed in duplicate. The higher of the 2 results is	658,596 258,731
10/5/2021	10/6/2021		222,782.20		This concentration was obtained using a pooled standard curve	906,442
10/5/2021		Port Richmond	93,091.28		This concentration was obtained using a pooled standard curve	226,167
10/5/2024	10/6/2021		134,813.58	54,670,565.39	This concentration was obtained using a pooled standard curve	224,029
10/5/2021			CO 472 00	A1 AE2 040 47	This concentration core allocational colors and a standard come	120,539
10/5/2021 10/5/2021 10/5/2021	10/6/2021	Rockaway Tallman Island	69,472.90 122,175.56		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907

10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	Concentration SARS- CoV-2 gene target (N1 Copies/L) 51,316.76 79,728.51 94,296.66 39,107.95 105,905.22 24,267.11 32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62 64,491.74	29,374,504.98 41,327,030.53 22,324,970.76 40,157,092.52 14,536,057.06 20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	Annotation This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve Concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection;This concentration This concentration was obtained using a pooled standard curve	Population Served, estimated 290,608 924,695 682,342 755,948 748,737 1,156,473 658,596 258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	51,316.76 79,728.51 94,296.66 39,107.95 105,905.22 24,267.11 32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62	population) 31,416,836.45 29,374,504.98 41,327,030.53 22,324,970.76 40,157,092.52 14,536,057.06 20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection; This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695 682,342 755,948 748,737 1,156,473 658,596 258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
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10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	79,728.51 94,296.66 39,107.95 105,905.22 24,267.11 32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62	29,374,504.98 41,327,030.53 22,324,970.76 40,157,092.52 14,536,057.06 20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection; This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342 755,948 748,737 1,156,473 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	94,296.66 39,107.95 105,905.22 24,267.11 32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	22,324,970.76 40,157,092.52 14,536,057.06 20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection;This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	682,342 755,948 748,737 1,156,473 658,596 258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
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10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Newtown Creek North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	24,267.11 32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	14,536,057.06 20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection; This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596 258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	North River Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	32,576.27 79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	20,034,521.74 28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve Concentration below Method Limit of Detection;This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596 258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Oakwood Beach Owls Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	79,603.82 51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	28,921,849.90 21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	Concentration below Method Limit of Detection; This concentration This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Owis Head Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve trins concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Port Richmond Red Hook Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	51,118.07 63,296.72 34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	21,389,376.61 25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167 224,029 120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/12/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Rockaway Tallman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	34,654.89 32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	25,668,539.47 22,854,361.83 14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/12/2021 1 10/17/2021 1	10/13/2021 10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Taliman Island Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	32,801.76 28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	14,351,279.73 15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907 1,201,485 290,608 924,695 682,342 755,948 748,737
10/12/2021 1 10/17/2021 1	10/13/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Wards Island 26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	28,326.37 51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	15,528,685.78 32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608 924,695 682,342 755,948 748,737
10/17/2021 10/17/2021	10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	26th Ward Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	51,450.78 184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	32,169,073.66 66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695 682,342 755,948 748,737
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Bowery Bay Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	184,044.14 110,923.66 32,570.15 149,890.62 184,160.62	66,300,849.37 43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342 755,948 748,737
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Coney Island Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	110,923.66 32,570.15 149,890.62 184,160.62	43,075,770.41 17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	682,342 755,948 748,737
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Hunts Point Jamaica Bay Newtown Creek North River Oakwood Beach	32,570.15 149,890.62 184,160.62	17,940,455.74 58,351,066.68	This concentration was obtained using a pooled standard curve	755,948 748,737
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021 10/18/2021	Jamaica Bay Newtown Creek North River Oakwood Beach	149,890.62 184,160.62	58,351,066.68		748,737
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021 10/18/2021	North River Oakwood Beach	,	101.873.423.93		
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021 10/18/2021	Oakwood Beach	64,491.74	- //	This concentration was obtained using a pooled standard curve	1,156,473
10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1 10/17/2021 1	10/18/2021 10/18/2021				this sample was analyzed in duplicate. The higher of the 2 results is	658,596
10/17/2021110/17/2021110/17/2021110/17/20211	10/18/2021		250,162.37		This concentration was obtained using a pooled standard curve	258,731
10/17/2021 1 10/17/2021 1 10/17/2021 1			323,893.32		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442
10/17/2021 1 10/17/2021 1	10/10/2021		53,106.91 137,505.14	, ,	This concentration was obtained using a pooled standard curve	226,167 224,029
10/17/2021 1	10/18/2021		42,482.85		This concentration was obtained using a pooled standard curve	120,539
10/17/2021 1			40,024.54		This concentration was obtained using a pooled standard curve	449,907
	10/18/2021	Wards Island	106,461.63	55,679,554.80	This concentration was obtained using a pooled standard curve	1,201,485
10/19/2021 1			79,519.23		This concentration was obtained using a pooled standard curve	290,608
10/19/2021 1		, ,	94,184.20		This concentration was obtained using a pooled standard curve	924,695
10/19/2021 1 10/19/2021 1			73,652.97 26,277.58		This concentration was obtained using a pooled standard curve	682,342 755,948
10/19/2021 1			65,827.31		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
		Newtown Creek	40,158.02		This concentration was obtained using a pooled standard curve	1,156,473
10/19/2021 1	10/20/2021	North River	40,038.13		This concentration was obtained using a pooled standard curve	658,596
10/19/2021 1	10/20/2021	Oakwood Beach	477,035.72	184,952,936.41	This concentration was obtained using a pooled standard curve	258,731
	10/20/2021		162,329.50		This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	88,747.53		This concentration was obtained using a pooled standard curve	226,167
10/19/2021 1 10/19/2021 1	10/20/2021		31,939.12 11,439.44		This concentration was obtained using a pooled standard curve Concentration below Method Limit of Quantification (above Method	224,029 120,539
10/19/2021 1			37,161.15		this sample was analyzed in duplicate. The higher of the 2 results is	449,907
10/19/2021 1			35,048.49		This concentration was obtained using a pooled standard curve	1,201,485
10/24/2021 1	10/25/2021	26th Ward	64,413.26	40,273,731.04	This concentration was obtained using a pooled standard curve	290,608
10/24/2021 1	10/25/2021	Bowery Bay	114,659.09	40,835,903.31	this sample was analyzed in duplicate. The higher of the 2 results is	924,695
10/24/2021 1			91,797.27	, ,	This concentration was obtained using a pooled standard curve	682,342
10/24/2021 1			20,482.91		This concentration was obtained using a pooled standard curve	755,948
10/24/2021 1			50,118.20		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	748,737
10/24/2021 1		Newtown Creek North River	98,128.73 44,471.76		This concentration was obtained using a pooled standard curve	1,156,473 658,596
		Oakwood Beach	82,972.94		This concentration was obtained using a pooled standard curve	258,731
10/24/2021 1			112,115.35		This concentration was obtained using a pooled standard curve	906,442
10/24/2021 1	10/25/2021	Port Richmond	107,171.76		This concentration was obtained using a pooled standard curve	226,167
10/24/2021 1			47,981.93		This concentration was obtained using a pooled standard curve	224,029
10/24/2021 1			56,257.47		This concentration was obtained using a pooled standard curve	120,539
10/24/2021 1 10/24/2021 1			58,542.71 61,218.19	25,120,780.97 32,788,684.41	This concentration was obtained using a pooled standard curve	449,907
10/24/2021 1			13,570.27		Concentration below Method Limit of Quantification (above Method	1,201,485 290,608
10/26/2021 1			21,227.59		This concentration was obtained using a pooled standard curve	924,695
10/26/2021 1			39,865.83		This concentration was obtained using a pooled standard curve	682,342
10/26/2021 1			23,247.52		This concentration was obtained using a pooled standard curve	755,948
10/26/2021 1			40,491.29		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	21,266.40		This concentration was obtained using a pooled standard curve	1,156,473
10/26/2021 1		North River Oakwood Beach	17,963.10		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	658,596
10/26/2021 1			102,561.30 43,556.27		This concentration was obtained using a pooled standard curve	258,731 906,442
		Port Richmond	22,312.94		This concentration was obtained using a pooled standard curve	226,167
10/26/2021 1			6,332.33		Concentration below Method Limit of Quantification (above Method	224,029
10/26/2021 1			26,661.85		This concentration was obtained using a pooled standard curve	120,539
10/26/2021 1			8,525.95		Concentration below Method Limit of Quantification (above Method	449,907
		Wards Island	18,497.74		This concentration was obtained using a pooled standard curve	1,201,485
	11/3/2021 11/3/2021		164,839.73 119,983.27		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per population)		estimated
11/2/2021	11/3/2021	Coney Island	55,367.46		This concentration was obtained using a pooled standard curve	682,342
11/2/2021		Hunts Point	59,277.73		This concentration was obtained using a pooled standard curve	755,948
11/2/2021		Jamaica Bay	89,875.15		This concentration was obtained using a pooled standard curve	748,737
11/2/2021		Newtown Creek	52,914.05		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
11/2/2021 11/2/2021		North River	35,011.78		This concentration was obtained using a pooled standard curve	658,596
11/2/2021		Oakwood Beach	89,705.76 112,321.58		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
11/2/2021		Port Richmond	31,535.12		This concentration was obtained using a pooled standard curve	226,167
11/2/2021	11/3/2021		162,916.05		This concentration was obtained using a pooled standard curve	224,029
11/2/2021	11/3/2021	Rockaway	18,358.94	12,683,982.34	This concentration was obtained using a pooled standard curve	120,539
11/2/2021		Tallman Island	57,502.07		This concentration was obtained using a pooled standard curve	449,907
11/2/2021		Wards Island	52,415.95		This concentration was obtained using a pooled standard curve	1,201,485
11/7/2021	11/8/2021		24,610.77		this sample was analyzed in duplicate. The higher of the 2 results is	290,608
11/7/2021 11/7/2021		Bowery Bay Coney Island	142,671.71 69,693.72		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	924,695 682,342
11/7/2021		Hunts Point	35,154.23		This concentration was obtained using a pooled standard curve	755,948
11/7/2021		Jamaica Bay	58,853.04		This concentration was obtained using a pooled standard curve	748,737
11/7/2021		Newtown Creek	49,202.90		This concentration was obtained using a pooled standard curve	1,156,473
11/7/2021		North River	51,496.01	29,894,324.88	This concentration was obtained using a pooled standard curve	658,596
11/7/2021		Oakwood Beach	90,208.87		This concentration was obtained using a pooled standard curve	258,731
11/7/2021			189,877.59		This concentration was obtained using a pooled standard curve	906,442
11/7/2021 11/7/2021		Port Richmond	75,597.68 75,383.60		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167
11/7/2021			7,050.14		Concentration below Method Limit of Quantification (above Method	120,539
11/7/2021		Tallman Island	84,710.44		This concentration was obtained using a pooled standard curve	449,907
11/7/2021		Wards Island	49,569.85	27,330,664.76	This concentration was obtained using a pooled standard curve	1,201,485
11/14/2021			48,627.50	31,670,674.38	This concentration was obtained using a pooled standard curve	290,608
	11/15/2021		97,464.36		This concentration was obtained using a pooled standard curve	924,695
	11/15/2021		106,269.53		This concentration was obtained using a pooled standard curve	682,342
	11/15/2021 11/15/2021		57,172.34 228,762.35		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
		Newtown Creek	83,595.88		This concentration was obtained using a pooled standard curve	1,156,473
	11/15/2021		63,664.19		This concentration was obtained using a pooled standard curve	658,596
11/14/2021	11/15/2021	Oakwood Beach	64,781.59	27,675,719.63	This concentration was obtained using a pooled standard curve	258,731
11/14/2021	11/15/2021	Owls Head	184,771.55	67,903,269.30	This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	128,395.66		This concentration was obtained using a pooled standard curve	226,167
11/14/2021			96,745.35		This concentration was obtained using a pooled standard curve	224,029
	11/15/2021	Tallman Island	33,552.03 100,720.51		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
		Wards Island	115,669.98		this sample was analyzed in duplicate. The higher of the 2 results is	1,201,485
	11/22/2021		108,054.36		This concentration was obtained using a pooled standard curve	290,608
11/21/2021	11/22/2021	Bowery Bay	330,982.76		This concentration was obtained using a pooled standard curve	924,695
11/21/2021	11/22/2021	Coney Island	103,197.32	42,937,871.12	This concentration was obtained using a pooled standard curve	682,342
	11/22/2021		70,361.28		This concentration was obtained using a pooled standard curve	755,948
	11/22/2021		163,031.08		This concentration was obtained using a pooled standard curve	748,737
	11/22/2021	Newtown Creek	110,069.30 139,322.10		This concentration was obtained using a pooled standard curve this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473 658,596
		Oakwood Beach	45,202.40		This concentration was obtained using a pooled standard curve	258,731
	11/22/2021		181,984.18		This concentration was obtained using a pooled standard curve	906,442
		Port Richmond	141,651.08		This concentration was obtained using a pooled standard curve	226,167
	11/22/2021		119,204.18		This concentration was obtained using a pooled standard curve	224,029
	11/22/2021		65,067.43		This concentration was obtained using a pooled standard curve	120,539
		Tallman Island	91,519.67		This concentration was obtained using a pooled standard curve	449,907
		Wards Island	128,936.55		This concentration was obtained using a pooled standard curve	1,201,485
	11/29/2021 11/29/2021		60,872.94 339,262.56		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695
	11/29/2021		265,869.08		this sample was analyzed in duplicate. The higher of the 2 results is	682,342
	11/29/2021	,	104,465.94		This concentration was obtained using a pooled standard curve	755,948
	11/29/2021		355,354.79		This concentration was obtained using a pooled standard curve	748,737
		Newtown Creek	134,571.16		This concentration was obtained using a pooled standard curve	1,156,473
	11/29/2021		215,095.96		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	97,956.52		This concentration was obtained using a pooled standard curve	258,731
	11/29/2021	Owis Head Port Richmond	211,802.74 211,865.07		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
	11/29/2021		138,299.65		This concentration was obtained using a pooled standard curve	226,167
	11/29/2021		35,158.68		This concentration was obtained using a pooled standard curve	120,539
11/20/2021		Tallman Island	163,369.21		This concentration was obtained using a pooled standard curve	449,907
	11/29/2021					4 204 405
11/28/2021 11/28/2021	11/29/2021	Wards Island	212,511.99	116,500,358.81	This concentration was obtained using a pooled standard curve	1,201,485
11/28/2021 11/28/2021 11/30/2021	11/29/2021 12/1/2021	26th Ward	497,132.71	317,302,706.21	this sample was analyzed in duplicate. The higher of the 2 results is	290,608
11/28/2021 11/28/2021	11/29/2021 12/1/2021 12/1/2021			317,302,706.21 140,096,491.86		

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
11/30/2021	12/1/2021	Jamaica Bay	308,060.19	population)	This concentration was obtained using a pooled standard curve	748,737
11/30/2021		Newtown Creek	270,985.41		This concentration was obtained using a pooled standard curve	1,156,473
11/30/2021		North River	177,786.21	, ,	This concentration was obtained using a pooled standard curve	658,596
11/30/2021	12/1/2021	Oakwood Beach	166,786.08	61,980,913.28	This concentration was obtained using a pooled standard curve	258,731
11/30/2021	12/1/2021		310,559.09		This concentration was obtained using a pooled standard curve	906,442
11/30/2021		Port Richmond	232,583.35		This concentration was obtained using a pooled standard curve	226,167
11/30/2021	12/1/2021		254,634.93		This concentration was obtained using a pooled standard curve	224,029
11/30/2021 11/30/2021	12/1/2021	Tallman Island	65,625.67 383,137.70		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
11/30/2021		Wards Island	241,057.16		This concentration was obtained using a pooled standard curve	1,201,485
12/5/2021	12/6/2021		101,371.61		This concentration was obtained using a pooled standard curve	290,608
12/5/2021	12/6/2021	Bowery Bay	613,214.21	288,685,165.02	This concentration was obtained using a pooled standard curve	924,695
12/5/2021		Coney Island	320,866.49		This concentration was obtained using a pooled standard curve	682,342
12/5/2021		Hunts Point	453,230.75		This concentration was obtained using a pooled standard curve	755,948
12/5/2021		Jamaica Bay	443,757.43		This concentration was obtained using a pooled standard curve	748,737
12/5/2021 12/5/2021		Newtown Creek North River	193,239.49 219,765.66		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,156,473 658,596
12/5/2021		Oakwood Beach	375,552.91		This concentration was obtained using a pooled standard curve	258,731
12/5/2021	12/6/2021		336,397.01		This concentration was obtained using a pooled standard curve	906,442
12/5/2021	12/6/2021	Port Richmond	294,892.69		This concentration was obtained using a pooled standard curve	226,167
12/5/2021	12/6/2021	Red Hook	74,078.64	40,054,529.99	This concentration was obtained using a pooled standard curve	224,029
12/5/2021	12/6/2021		101,320.68		This concentration was obtained using a pooled standard curve	120,539
12/5/2021		Tallman Island	213,245.44		this sample was analyzed in duplicate. The higher of the 2 results is	449,907
12/5/2021		Wards Island	137,974.38		This concentration was obtained using a pooled standard curve	1,201,485
12/7/2021 12/7/2021	12/8/2021	Bowery Bay	146,892.68 244,576.41		This concentration was obtained using a pooled standard curve	290,608 924,695
12/7/2021		Coney Island	229,093.33		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	682,342
12/7/2021		Hunts Point	133,105.67		This concentration was obtained using a pooled standard curve	755,948
12/7/2021		Jamaica Bay	232,934.02		This concentration was obtained using a pooled standard curve	748,737
12/7/2021		Newtown Creek	368,691.79		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
12/7/2021	12/8/2021	North River	173,401.23	95,679,135.11	This concentration was obtained using a pooled standard curve	658,596
12/7/2021	12/8/2021	Oakwood Beach	592,428.95	227,958,860.16	This concentration was obtained using a pooled standard curve	258,731
12/7/2021	12/8/2021		405,634.61		This concentration was obtained using a pooled standard curve	906,442
12/7/2021		Port Richmond	368,593.30		This concentration was obtained using a pooled standard curve	226,167
12/7/2021	12/8/2021		145,801.88		This concentration was obtained using a pooled standard curve	224,029
12/7/2021 12/7/2021	12/8/2021	Tallman Island	90,347.41 364,639.69		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
12/7/2021		Wards Island	423,493.20		This concentration was obtained using a pooled standard curve	1,201,485
12/12/2021	12/13/2021		95,535.25		This concentration was obtained using a pooled standard curve	290,608
12/12/2021	12/13/2021		391,351.88		This concentration was obtained using a pooled standard curve	924,695
12/12/2021	12/13/2021	Coney Island	549,942.69	228,817,647.51	This concentration was obtained using a pooled standard curve	682,342
12/12/2021			164,715.10		This concentration was obtained using a pooled standard curve	755,948
12/12/2021	12/13/2021		447,936.79	, ,	This concentration was obtained using a pooled standard curve	748,737
12/12/2021		Newtown Creek	273,633.40		This concentration was obtained using a pooled standard curve	1,156,473
	12/13/2021	North River Oakwood Beach	54,931.89		This concentration was obtained using a pooled standard curve	658,596
	12/13/2021		321,991.95 732,265.13		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	258,731 906,442
12/12/2021		Port Richmond	431,163.55		This concentration was obtained using a pooled standard curve	226,167
12/12/2021			299,794.85		this sample was analyzed in duplicate. The higher of the 2 results is	224,029
	12/13/2021		91,375.86		This concentration was obtained using a pooled standard curve	120,539
12/12/2021		Tallman Island	566,127.91		This concentration was obtained using a pooled standard curve	449,907
12/12/2021		Wards Island	62,398.50		This concentration was obtained using a pooled standard curve	1,201,485
	12/15/2021		356,498.02		This concentration was obtained using a pooled standard curve	290,608
	12/15/2021		536,429.96	, ,	This concentration was obtained using a pooled standard curve	924,695
	12/15/2021		597,777.24		This concentration was obtained using a pooled standard curve	682,342
12/14/2021 12/14/2021			158,138.40 412,824.05		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
12/14/2021		Newtown Creek	412,824.03		This concentration was obtained using a pooled standard curve	1,156,473
12/14/2021			300,698.89		This concentration was obtained using a pooled standard curve	658,596
12/14/2021		Oakwood Beach	151,840.99		This concentration was obtained using a pooled standard curve	258,731
12/14/2021	12/15/2021		448,429.99		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
12/14/2021		Port Richmond	676,765.62		This concentration was obtained using a pooled standard curve	226,167
			430,229.02		This concentration was obtained using a pooled standard curve	224,029
12/14/2021			508,900.48		This concentration was obtained using a pooled standard curve	120,539
12/14/2021		Tallman Island	272,111.74		This concentration was obtained using a pooled standard curve	449,907
12/14/2021		Wards Island	458,344.03		This concentration was obtained using a pooled standard curve	1,201,485
	12/20/2021 12/20/2021		705,606.61 296,116.50		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608 924,695
	12/20/2021		778,698.74		This concentration was obtained using a pooled standard curve	682,342
	,,					
12/19/2021	12/20/2021	Hunts Point	346,140.13	202,795,732.80	This concentration was obtained using a pooled standard curve	755,948
			346,140.13 1,150,708.16		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1 copies per day per	Annotation	Served,
			(N1 Copies/L)	population)		estimated
12/19/2021	12/20/2021	North River	800,732.86		This concentration was obtained using a pooled standard curve	658,596
12/19/2021		Oakwood Beach	914,508.26		This concentration was obtained using a pooled standard curve	258,731
12/19/2021			1,238,978.46		This concentration was obtained using a pooled standard curve	906,442
12/19/2021 12/19/2021		Port Richmond	1,640,761.43 849,697.47		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	226,167 224,029
12/19/2021			497,179.24		This concentration was obtained using a pooled standard curve	120,539
12/19/2021		Tallman Island	391,817.26		This concentration was obtained using a pooled standard curve	449,907
12/19/2021	12/20/2021	Wards Island	863,853.38		This concentration was obtained using a pooled standard curve	1,201,485
12/26/2021	12/27/2021		4,348,245.43	2,718,695,986.10	This concentration was obtained using a pooled standard curve	290,608
12/26/2021	12/27/2021		4,926,373.34		This concentration was obtained using a pooled standard curve	924,695
12/26/2021		Coney Island	2,364,219.23	, ,	This concentration was obtained using a pooled standard curve	682,342
12/26/2021	12/27/2021 12/27/2021		4,463,091.68 4,940,260.69		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
12/26/2021		Newtown Creek	3,353,162.97		This concentration was obtained using a pooled standard curve	1,156,473
12/26/2021	12/27/2021		2,798,388.43		This concentration was obtained using a pooled standard curve	658,596
12/26/2021	12/27/2021	Oakwood Beach	2,232,671.64	836,236,808.31	This concentration was obtained using a pooled standard curve	258,731
12/26/2021	12/27/2021		2,750,579.08		This concentration was obtained using a pooled standard curve	906,442
12/26/2021		Port Richmond	4,128,726.49		this sample was analyzed in duplicate. The higher of the 2 results is	226,167
12/26/2021	12/27/2021		2,268,140.61		This concentration was obtained using a pooled standard curve	224,029
12/26/2021 12/26/2021	12/27/2021	Tallman Island	1,601,033.26 1,932,399.23		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539 449,907
12/26/2021		Wards Island	4,590,275.31	, ,	This concentration was obtained using a pooled standard curve	1,201,485
1/2/2022		26th Ward	870,953.96		This concentration was obtained using a pooled standard curve	290,608
1/2/2022	1/3/2022	Bowery Bay	2,135,905.49	926,834,761.35	This concentration was obtained using a pooled standard curve	924,695
1/2/2022		Coney Island	2,155,789.66	1,016,567,291.03	This concentration was obtained using a pooled standard curve	682,342
1/2/2022		Hunts Point	2,330,782.44		This concentration was obtained using a pooled standard curve	755,948
1/2/2022		Jamaica Bay	2,197,077.09		This concentration was obtained using a pooled standard curve	748,737
1/2/2022 1/2/2022		Newtown Creek North River	1,559,631.63 1,673,006.17		this sample was analyzed in duplicate. The higher of the 2 results is This concentration was obtained using a pooled standard curve	1,156,473 658,596
1/2/2022		Oakwood Beach	1,080,416.91		This concentration was obtained using a pooled standard curve	258,731
1/2/2022		Owls Head	2,888,112.89		This concentration was obtained using a pooled standard curve	906,442
1/2/2022	1/3/2022	Port Richmond	2,512,053.13	1,219,299,811.15	This concentration was obtained using a pooled standard curve	226,167
1/2/2022		Red Hook	1,058,910.89		This concentration was obtained using a pooled standard curve	224,029
1/2/2022		Rockaway	2,960,280.55		This concentration was obtained using a pooled standard curve	120,539
1/2/2022		Tallman Island	2,364,616.87		This concentration was obtained using a pooled standard curve	449,907
1/2/2022 1/4/2022		Wards Island 26th Ward	2,928,422.16 1,706,211.30		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	1,201,485 290,608
1/4/2022		Bowery Bay	2,008,471.39		this sample was analyzed in duplicate. The higher of the 2 results is	924,695
1/4/2022		Coney Island	1,426,396.08		This concentration was obtained using a pooled standard curve	682,342
1/4/2022	1/5/2022	Hunts Point	1,882,738.13	1,197,332,145.30	This concentration was obtained using a pooled standard curve	755,948
1/4/2022		Jamaica Bay	3,008,367.21		This concentration was obtained using a pooled standard curve	748,737
1/4/2022		Newtown Creek	1,536,469.59		This concentration was obtained using a pooled standard curve	1,156,473
1/4/2022		North River	1,204,266.38 1,427,283.14		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	658,596
1/4/2022 1/4/2022		Oakwood Beach Owls Head	3,163,300.98		This concentration was obtained using a pooled standard curve	258,731 906,442
1/4/2022		Port Richmond	1,811,175.64		This concentration was obtained using a pooled standard curve	226,167
1/4/2022		Red Hook	1,194,055.83		This concentration was obtained using a pooled standard curve	224,029
1/4/2022	1/5/2022	Rockaway	877,956.05	606,569,816.80	This concentration was obtained using a pooled standard curve	120,539
1/4/2022		Tallman Island	1,638,613.79	, ,	This concentration was obtained using a pooled standard curve	449,907
1/4/2022		Wards Island	1,904,525.26		This concentration was obtained using a pooled standard curve	1,201,485
1/10/2022	1/11/2022		275,653.90		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	290,608
1/10/2022 1/10/2022		Bowery Bay Coney Island	843,828.38 1,286,343.54		original RT-qPCR failed, RT-qPCR repeated;This concentration was	924,695 682,342
1/10/2022		Hunts Point	469,871.16		This concentration was obtained using a pooled standard curve	755,948
1/10/2022		Jamaica Bay	1,790,990.67		This concentration was obtained using a pooled standard curve	748,737
1/10/2022		Newtown Creek	210,856.20		This concentration was obtained using a pooled standard curve	1,156,473
1/10/2022		North River	815,409.69		this sample was analyzed in duplicate. The higher of the 2 results is	658,596
1/10/2022		Oakwood Beach	714,577.58		This concentration was obtained using a pooled standard curve	258,731
1/10/2022 1/10/2022	1/11/2022	Owls Head Port Richmond	1,365,795.45		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
1/10/2022	1/11/2022		818,122.57 855,152.85		This concentration was obtained using a pooled standard curve	226,167
1/10/2022	1/11/2022		756,962.01		This concentration was obtained using a pooled standard curve	120,539
1/10/2022		Tallman Island	1,515,713.41		This concentration was obtained using a pooled standard curve	449,907
1/10/2022	1/11/2022	Wards Island	1,328,758.11		This concentration was obtained using a pooled standard curve	1,201,485
1/12/2022	1/13/2022		340,835.80		This concentration was obtained using a pooled standard curve	290,608
1/12/2022		Bowery Bay	1,344,537.66		This concentration was obtained using a pooled standard curve	924,695
1/12/2022		Coney Island	1,122,840.55		This concentration was obtained using a pooled standard curve	682,342
1/12/2022 1/12/2022		Hunts Point Jamaica Bay	755,035.85		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	755,948 748,737
1/12/2022		Newtown Creek	787,867.56		This concentration was obtained using a pooled standard curve	1,156,473
1/12/2022		North River	590,016.22		This concentration was obtained using a pooled standard curve	658,596
		Oakwood Beach	481,770.56	106 700 605 47	This concentration was obtained using a pooled standard curve	258,731

			Concentration SARS-	Per capita SARS-		Population
Sample Date	Test date	WRRF Name	CoV-2 gene target	CoV-2 load (N1	Annotation	Served,
			(N1 Copies/L)	copies per day per		estimated
1/12/2022	1/13/2022	Owls Head	1,584,243.67	582 207 179 29	This concentration was obtained using a pooled standard curve	906,442
1/12/2022		Port Richmond	977,131.80		This concentration was obtained using a pooled standard curve	226,167
1/12/2022	1/13/2022		421,779.24	, ,	This concentration was obtained using a pooled standard curve	220,107
1/12/2022	1/13/2022		319,068.34		this sample was analyzed in duplicate. The higher of the 2 results is	120,539
1/12/2022		Tallman Island	1,156,588.28		This concentration was obtained using a pooled standard curve	449,907
1/12/2022		Wards Island	766,146.44		This concentration was obtained using a pooled standard curve	1,201,485
1/17/2022	1/8/2022	26th Ward	121,460.97	148,720,196.40	This concentration was obtained using a pooled standard curve	290,608
1/17/2022	1/8/2022	Bowery Bay	204,235.30	122,903,019.59	This concentration was obtained using a pooled standard curve	924,695
1/17/2022	1/8/2022	Coney Island	409,861.85	247,841,945.63	this sample was analyzed in duplicate. The higher of the 2 results is	682,342
1/17/2022		Hunts Point	51,418.83		This concentration was obtained using a pooled standard curve	755,948
1/17/2022		Jamaica Bay	338,905.79		This concentration was obtained using a pooled standard curve	748,737
1/17/2022		Newtown Creek	179,751.25		This concentration was obtained using a pooled standard curve	1,156,473
1/17/2022		North River	61,134.86		This concentration was obtained using a pooled standard curve	658,596
1/17/2022 1/17/2022		Oakwood Beach	310,724.60	, ,	This concentration was obtained using a pooled standard curve	258,731
1/17/2022		Owls Head Port Richmond	368,833.48 251,460.20		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	906,442 226,167
1/17/2022		Red Hook	149,567.64		This concentration was obtained using a pooled standard curve	220,107
1/17/2022		Rockaway	215,085.66		This concentration was obtained using a pooled standard curve	120,539
1/17/2022		Tallman Island	231,120.55		This concentration was obtained using a pooled standard curve	449,907
1/17/2022		Wards Island	283,328.22		This concentration was obtained using a pooled standard curve	1,201,485
1/19/2022	1/20/2022	26th Ward	259,269.06		This concentration was obtained using a pooled standard curve	290,608
1/19/2022	1/20/2022	Bowery Bay	384,522.16	146,392,467.99	This concentration was obtained using a pooled standard curve	924,695
1/19/2022	1/20/2022	Coney Island	514,415.99	199,766,804.22	This concentration was obtained using a pooled standard curve	682,342
1/19/2022	1/20/2022	Hunts Point	124,567.41	, ,	This concentration was obtained using a pooled standard curve	755,948
1/19/2022		Jamaica Bay	452,300.09		This concentration was obtained using a pooled standard curve	748,737
1/19/2022		Newtown Creek	338,956.59		This concentration was obtained using a pooled standard curve	1,156,473
1/19/2022		North River	365,593.51	, ,	This concentration was obtained using a pooled standard curve	658,596
1/19/2022		Oakwood Beach	333,408.67		This concentration was obtained using a pooled standard curve	258,731
1/19/2022	1/20/2022		716,066.45		this sample was analyzed in duplicate. The higher of the 2 results is	906,442
1/19/2022 1/19/2022	1/20/2022	Port Richmond	339,904.20 278,200.51		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	226,167
1/19/2022	1/20/2022		232,248.29		This concentration was obtained using a pooled standard curve	120,539
1/19/2022		Tallman Island	324,814.92		This concentration was obtained using a pooled standard curve	449,907
1/19/2022		Wards Island	448,001.28		This concentration was obtained using a pooled standard curve	1,201,485
1/24/2022	1/25/2022		150,114.11		This concentration was obtained using a pooled standard curve	290,608
1/24/2022	1/25/2022	Bowery Bay	317,077.04		This concentration was obtained using a pooled standard curve	924,695
1/24/2022	1/25/2022	Coney Island	308,116.32	126,490,304.38	This concentration was obtained using a pooled standard curve	682,342
1/24/2022	1/25/2022	Hunts Point	193,125.08	134,423,274.70	This concentration was obtained using a pooled standard curve	755,948
1/24/2022	1/25/2022	Jamaica Bay	216,340.46	82,031,876.33	This concentration was obtained using a pooled standard curve	748,737
1/24/2022		Newtown Creek	197,504.65		this sample was analyzed in duplicate. The higher of the 2 results is	1,156,473
1/24/2022		North River	173,719.00		This concentration was obtained using a pooled standard curve	658,596
1/24/2022		Oakwood Beach	272,850.13		This concentration was obtained using a pooled standard curve	258,731
1/24/2022	1/25/2022		464,230.01		This concentration was obtained using a pooled standard curve	906,442
1/24/2022		Port Richmond	207,595.94		This concentration was obtained using a pooled standard curve	226,167 224,029
1/24/2022 1/24/2022	1/25/2022 1/25/2022		268,015.34 116,504.90		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	120,539
1/24/2022		Tallman Island	328,269.46		This concentration was obtained using a pooled standard curve	449,907
1/24/2022		Wards Island	108,361.21		This concentration was obtained using a pooled standard curve	1,201,485
1/24/2022	1/23/2022		88,303.49		This concentration was obtained using a pooled standard curve	290,608
1/26/2022		Bowery Bay	169,389.31	22,200,020.00	No flow data; This concentration was obtained using a pooled	924,695
1/26/2022		Coney Island	212,714.43	85,072,759.70	This concentration was obtained using a pooled standard curve	682,342
1/26/2022		Hunts Point	138,729.37		This concentration was obtained using a pooled standard curve	755,948
1/26/2022		Jamaica Bay	181,652.70		No flow data; This concentration was obtained using a pooled	748,737
1/26/2022	1/27/2022	Newtown Creek	88,841.03		This concentration was obtained using a pooled standard curve	1,156,473
1/26/2022		North River	112,692.33		This concentration was obtained using a pooled standard curve	658,596
1/26/2022		Oakwood Beach	133,448.63		This concentration was obtained using a pooled standard curve	258,731
1/26/2022		Owls Head	318,159.09	118,402,202.13	This concentration was obtained using a pooled standard curve	906,442
1/26/2022		Port Richmond	249,040.15		this sample was analyzed in duplicate. The higher of the 2 results is	226,167
1/26/2022	1/27/2022		167,723.39		This concentration was obtained using a pooled standard curve	224,029
1/26/2022	1/27/2022		118,225.33		This concentration was obtained using a pooled standard curve	120,539
1/26/2022		Tallman Island	242,389.85		This concentration was obtained using a pooled standard curve This concentration was obtained using a pooled standard curve	449,907
1/26/2022	1/2//2022	Wards Island	147,350.28	32,323,378.52	This concentration was obtained using a pooled standard curve	1,201,485