# Supplementary Materials for:

# Assessment of a SARS-CoV-2 wastewater monitoring program in El Paso, Texas from November 2020 to June 2022

## Methods for Molecular Analysis (adapted from Petrosino et al. 2020)

## *Virus Concentration*

From each sample, 200 mL was centrifuged at 10,000g for 4 min at 4°C to remove large debris and sludge from the wastewater sample. Next, 50 mL of supernatant from each sample was poured into the 6-head (EZFITMVHE3, MilliporeSigma) EZ Fit Manifold (EZFITBASE6, MilliporeSigma) vacuum system. MgCl2\*6H2O was added to achieve a 25 mM concentration. After homogenizing each sample by swirling a pipette tip (and allowing the sample to rest for 5 minutes), the sample was then pulled through the filter by a vacuum pump (Petrosino et al. 2020). After filtration, the filter was folded and placed into a bead tube with 0.1mm glass beads. The tubes were stored at -80°C and permitted to freeze before bead beating and nucleic acid extraction.

## *Nucleic acid extraction*

For the viral RNA extraction in each wastewater sample, 300 μL was extracted according to the manufacturer’s instruction associated with the chemagic Viral DNA/RNA 300 kit special H96 (CMG-1033-S, Perkin Elmer) along with the chemagic 360 (2024-0020, Perkin Elmer) automated platform (Petrosino et al. 2020). The extraction was then eluted in 100 μL sterile, nuclease free water and stored at -80°C until quantification.

## *SARS-CoV-2 RNA quantification*

Samples were analyzed using the CDC 2019-Novel coronavirus (2019-ncoV) Real-Time reverse transcription-polymerase chain reaction (RT-PCR) Diagnostic panel. The nucleocapsid (N) gene of the SARS-CoV-2 genome is targeted in this specific assay (N1 and N2). Real-Time RT-PCR was performed for all samples using 10 μL of eluted RNA and 15 μL of TaqPath 1-step RT-PCR Master Mix, CG (A15299 Applied Biosystems). Cycling conditions were as follows: 25°C for 2 minutes, 50°C for 15 minutes, 95°C for 2 minutes, and 45 cycles of 95°C for 3 seconds, 55°C for 30 seconds on a 7500 Fast Dx Real-Time PCR Instrument (4406985, Applied Biosystems) with SDS version 1.4 software (Petrosino et al. 2020). For the Real-Time RT-PCR methods, negative extraction, no template negative controls, and a standard curve of the linearized N plasmid were used to identify the genomic copy numbers for both N1 and N2. In this analysis, we focused specifically on the SARS-CoV-2 concentrations using the N1 assay.

***RNA quantification quality control***

The standard curve ranged from 10,000-16 genomic copies/mL with N1 primer values of: = 0.992 and efficiency = 99.1% (Petrosino et al. 2020). The limit of detection (LOD) was set as 2 gene copies/10 μL RNA template, and after converting μL to liters in wastewater (with a 30x concentration factor from wastewater to RNA extract), the LOD was identified as 6,667 copies/L wastewater. Samples with a cycle threshold lower than 40 were considered positive.

Chart

Description automatically generated

Figure S1. Total number of wastewater samplings per month in the study region.

Diagram

Description automatically generated

Figure S2. Number of wastewater samplings per month for each WWTF.

A picture containing text, map, indoor

Description automatically generated

Figure S3. Time series of wastewater SARS-CoV-2 RNA concentration (copies/L) in 10k for each of the four WWTFs. N1 and N2 refer to the different primer-probe targets used for SARS-CoV-2 quantification in wastewater.

Diagram, engineering drawing

Description automatically generated

Figure S4. Panel plot showing the COVID-19 case rate and wastewater concentrations for each WWTF (left panel: original scale; right panel: log scale).