Project Title: El Paso Waters

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Materials and Methods

Virus Concentration

Primary wastewater was sampled weekly from four El Paso wastewater treatment plants from May 2020 to present. Wastewater samples were collected with 0.5 L polypropylene bottles and shipped in a cooler box with ice to the Alkek Center for Metagenomics and Microbiome Research (CMMR) at Baylor College of Medicine. Samples were stored at 4 °C and used within one week of collection.

SARS-CoV-2 in wastewater samples were concentrated using a PEG precipitation method for the samples collected from May 22nd and November 5th, 2020. These samples were aliquoted into 250 mL duplicates and then centrifuged at 13,000 *g* for 15 minutes at 4 °C in 500 mL polypropylene bottles to remove sludge and large debris. Supernatants from duplicates were combined and passed through 0.22 µm filters (SCGPS05RE, MilliporeSigma) using the Stericup® vacuum filtration system unit (MiliporeSigma©). The filtered sample was mixed thoroughly with PEG (8% w/v, 16 g) and NaCl (0.5 M, 5.844 g), and incubated overnight at 4 °C. After incubation, the sample was centrifuged at 20,000 *g* for 30 min at 4 °C and supernatant was carefully discarded. Pellets were resuspended in 1 mL 1X PBS solution and stored at −80°C until further analysis.

The concentration method for SARS-CoV-2 in wastewater samples from November 20th, 2020 until the most recently collected sample was substituted by the electronegative filtration method. This method was performed at the Tailored Antibacterials and Innovative Laboratories for Phage Research at Baylor College of Medicine. A total of 200 mL of wastewater was centrifuged from each sample at 10,000 g for 4 min at 4 °C to remove sludge and large debris. Subsequently, 50 mL of supernatant was poured into the 6-head (EZFITMVHE3, MilliporeSigma) EZ Fit Manifold (EZFITBASE6, MilliporeSigma) vacuum system followed by the addition of $MgCl2*6H_2O$ to achieve a final concentration of 25 mM. The samples were gently swirled with a pipette tip to homogenize and allowed to sit for five minutes. A vacuum pump then pulled the sample through the filter. After filtration was complete, the filter was folded and placed into a bead tube containing 0.1 mm glass beads. Bead tubes containing filters were stored at −80 °C and allowed to freeze prior to bead beating and nucleic acid extraction.

Nucleic Acid Extraction

Viral RNA from wastewater eluates and precipitates was extracted using the QIAamp Viral Mini RNA Kit (52906, Qiagen) with the QIAcube Connect (9002864, Qiagen) automated platform, according to

manufacturer instructions. 140 µl of wastewater was extracted and eluted with 100 µl of elution buffer (May 22nd and November 5th, 2020).

After November 20th, 2020, viral RNA was extracted using chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033-S, Perkin Elmer) with the chemagic 360 (2024-0020, Perkin Elmer) automated platform. 300 µl of each sample was extracted according to manufacturer instructions and eluted in 100 µl sterile, nuclease-free water. All extracts were stored at −80 °C until quantification.

QUANTIFICATION OF SARS-COV-2 RNA

The extracted RNA was tested using the CDC 2019-Novel coronavirus (2019-ncoV) Real-Time RT-PCR Diagnostic panel (28). The assay targets the nucleocapsid (N) gene (N1 and N2) of the SARS-CoV-2 genome. Real-time RT-PCR was performed using 10 µl of eluted RNA and 15 µl of TaqPath 1-step RT-PCR Master Mix, CG (A15299 Applied Biosystems) under the following cycling conditions: 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. Samples were considered positive if N1, N2, Ct values were <40. The real-time RT-PCR included negative extraction, no template negative controls, and a standard curve of the linearized N plasmid to determine the genomic copy numbers of N1 and N2 in the samples. The standard curve ranged from 10,000-16 copies/mL with N1 primer values of R2: 0.992, efficiency: 99.1% and N2 primer values of R2: 0.969, efficiency: 97.4%. Limit of detection (LOD) was set as 2 gene copies/10µl RNA template. Applying a concentration factor of 30x from wastewater to RNA extract, and converting from µL to L, the LOD was calculated as 6,667 copies/L wastewater.

DATA ANALYSIS

All data were managed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). All samples with cycle threshold (Ct) larger than 40 were considered negative. All electronic data that was updated weekly consisted of wastewater analysis results (N1 and N2 copies/L of wastewater), where flow rate information was obtained from the City of El Paso.

STATISTICAL METHODS AND MODEL

Successful detection of SARS-COV-2 in wastewater suggests the potential utility of wastewater-based epidemiology (WBE) for COVID-19 community surveillance. In order to achieve this objective, the clinical positivity rate from the City of El Paso should be obtained to allow data correlation with weekly viral load estimates.