Wastewater Informed Epidemiological Monitoring for SARS-CoV-2 in Bexar County, Texas

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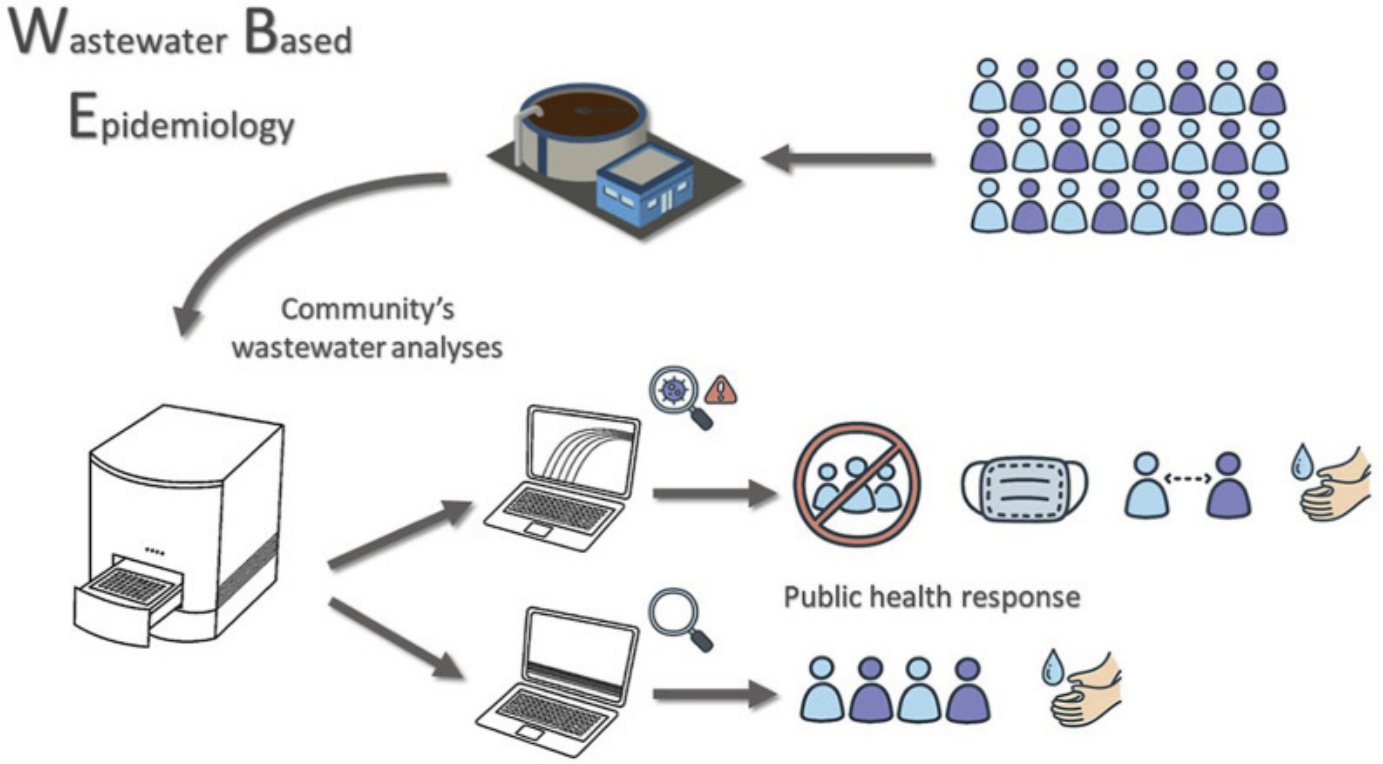
WASTEWATER-BASED EPIDEMIOLOGY (WBE)

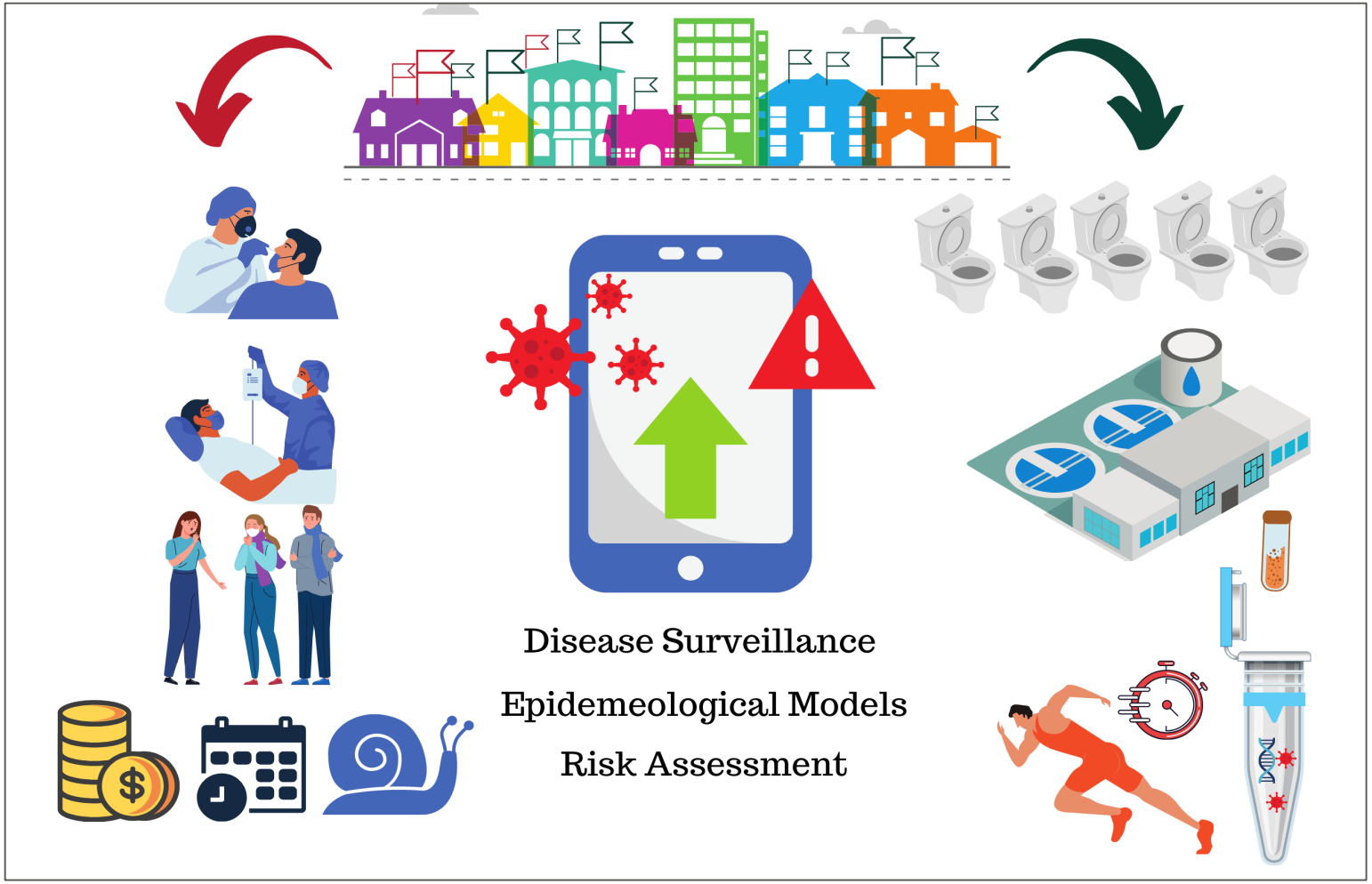
- An innovative epidemiology tool that enables information about exposure to pathogens and disease-causing agents retrieved from wastewater via the analysis of human metabolic excretion products (called biomarkers).
- A complementary approach for current infectious disease surveillance systems.
- An early warning system for disease outbreaks.
- Wastewater testing for evidence of pathogens is not new – poliovirus and antimicrobial resistance



CLINICAL DATA ALONE

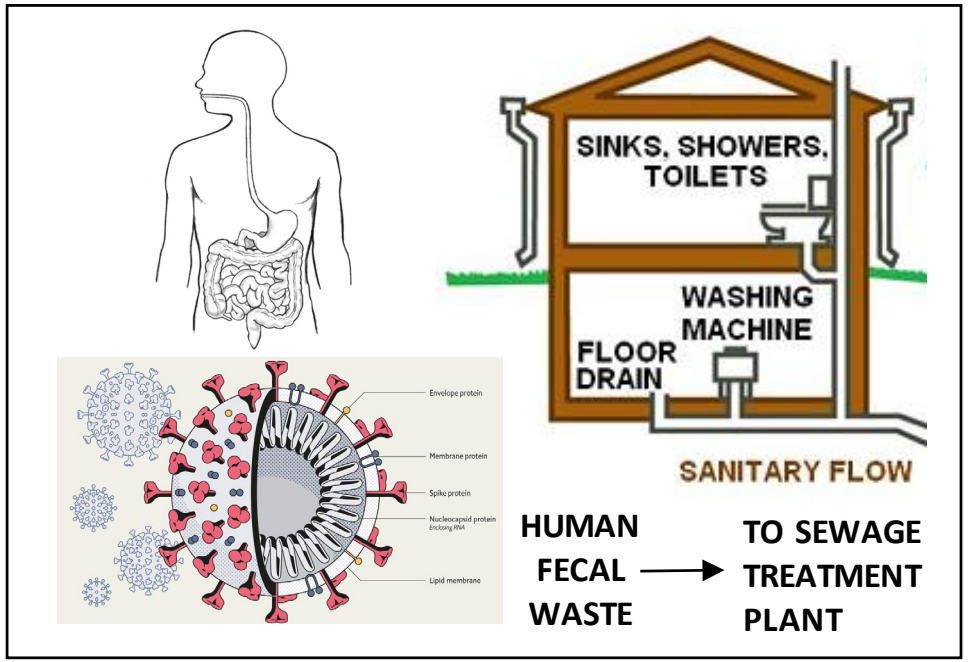
VS.

CLINICAL DATA SUPPORTED BY WBE



WBE FOR SARS-COV-2

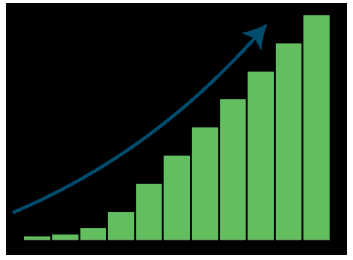
- Studies have demonstrated the presence of viral RNA in fecal samples of SARS-CoV-2 infected individuals.
- The amount of RNA excreted is variable from one patient to another, but the signal can be detected in asymptomatic individuals too.
- The genetic material of SARS-CoV-2 makes it a suitable candidate to serve as a WBE biomarker.



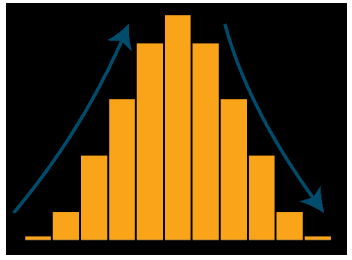
Stable → Quantifiable for downstream detection → Unique to SARS-CoV-2

USES OF WBE DATA

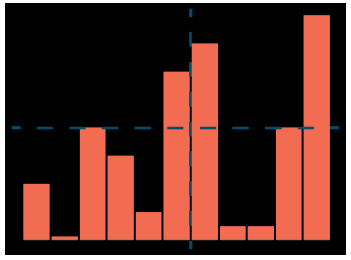
General Use Cases	Can Inform
Assess Level of Community Infection	Tracking disease prevalence in the community. Identification of “hot spots” and areas that are not impacted by the virus
Trends/Changes in Infection	Early detection of disease. Tracking the impact of medical and social interventions. Leading indicator of community infection.
Risk Assessment	Estimate of the percentage of mild COVID-19 presence. Complement clinical data for decision making.
Viral Evolution	Source tracking of the virus.



Trend occurrence



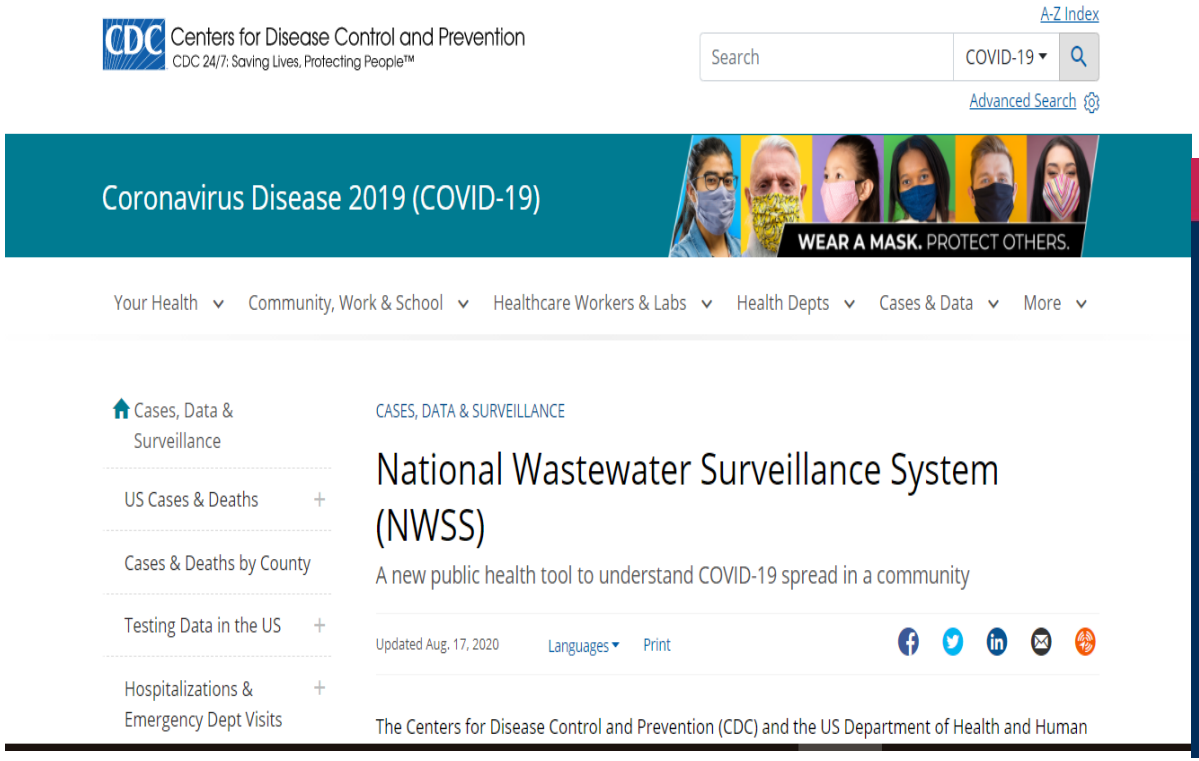
Changes in Trends



Community Prevalence

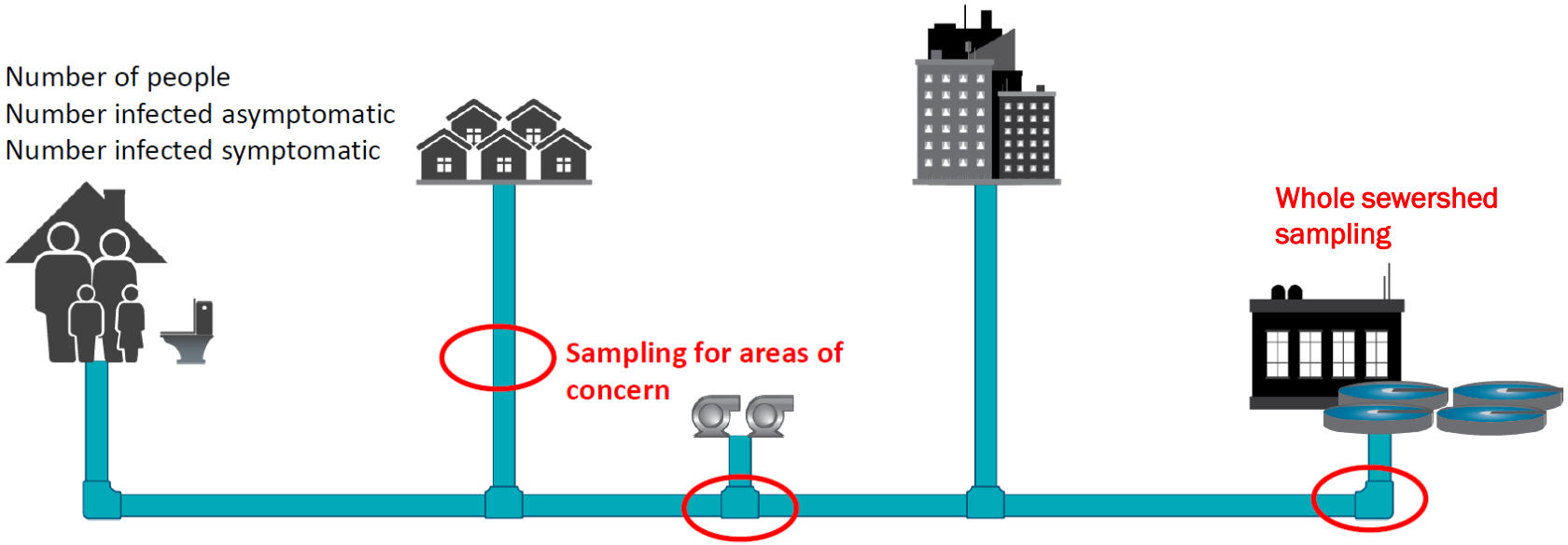
USE CASES OF WASTEWATER SURVEILLANCE

Federal agencies: CDC's National Wastewater Surveillance Database for COVID-19



APPROACH

- Raw sewage samples collected from the wastewater treatment plants and/or lift stations
- Develop, evaluate and apply methods for concentrating and quantifying SARS-CoV-2 in wastewater by molecular assays



SAFETY CONSIDERATIONS

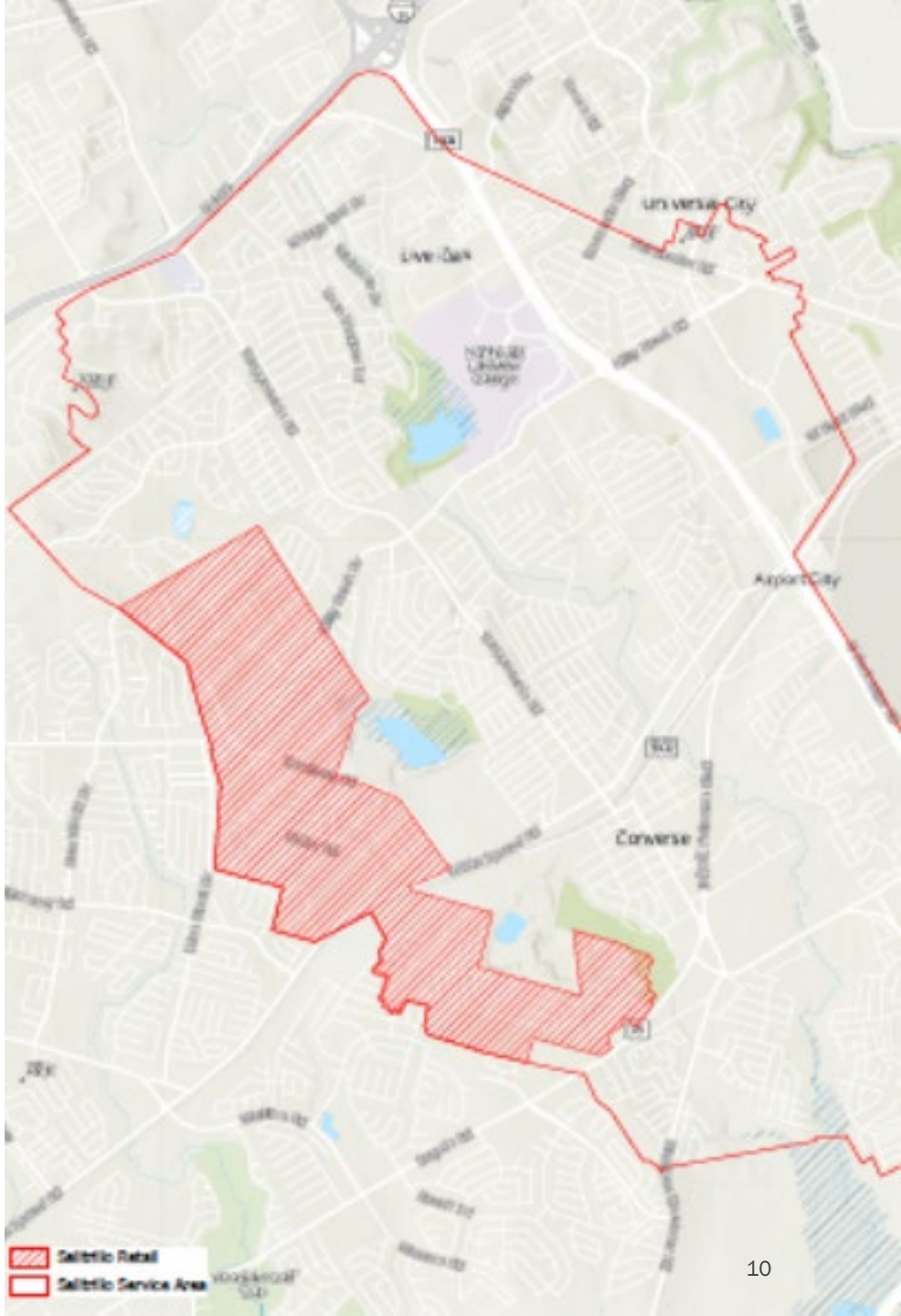
- To date, no infectious SARS-CoV2 virus has been recovered from untreated or treated sewage
- Given the myriad pathogens routinely expected to be found in untreated sewage and the commensurate precautions normally taken, sewage sampling in the context of COVID-19 is not expected to engender any additional infection risk to workers
- Laboratory processing of wastewater samples should follow existing biosafety standards at BSL-2

PILOT STUDY

- Screening of SARS-CoV-2 genetic material presence in untreated sewage within selected wastewater treatment plants in Bexar County, Texas.
- Development of a robust molecular technique to extract and quantify viral RNA related to SARS-CoV-2 in sewage samples.
- Correlating viral RNA concentrations in wastewater samples collected with trends of cases within the same area.
- Communicating wastewater surveillance results with the health officials, the City, wastewater workers, and the public.

METHODOLOGY

- **Sample collection and processing**
- Raw sewage samples are collected from the Salitrillo WWTP operated by San Antonio River Authority (SARA).
- The Salitrillo WWTP has approximately 17,000 connections with an average daily flow of 4.859 MGD and a permitted flow of 5.83 MGD.
- 24hr composite influent sewage samples (2 L) collected weekly using ISCO autosampler. Effluent samples also collected using grab method.
- Samples are transported on ice to the laboratory at UTSA and characterized for standard water quality parameters (e.g. COD, BOD, pH, nutrients, cations & anions).



METHODOLOGY

- **Sample collection and processing**
- The samples are acidified with 2N hydrochloric acid to reach a pH of 3 – 4
- 200 mL of sample filtered through a 0.45 µm GN-6 Metricel membrane to concentrate viral RNA by electronegative adsorption.
- Each sample is filtered into four replicate membrane filters. ~800 mL total volume was filtered.

METHODOLOGY

- **RNA Extraction**
- RNA extraction of membrane filters with adsorbed viral RNA was done using the RNeasy Power Microbiome Kit (Qiagen) and in combination with automated robot Qiacube Connect according to the manufacturer's Standard with DNase protocol.
- The concentration and purity of extracted RNA was measured using nanodrop. RNA extracts were stored at −80 °C for subsequent molecular analyses.

METHODOLOGY

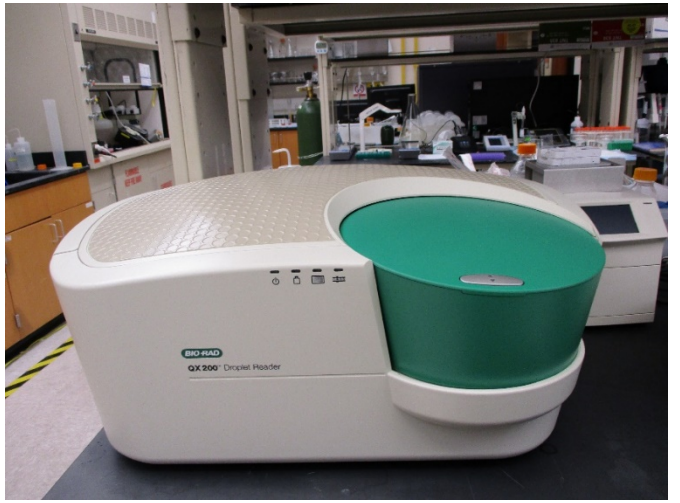
- Quantification of SARS-CoV-2 viral RNA
- For RNA detection and quantification, one-step reverse transcriptase droplet digital polymerase chain reaction (**RT-ddPCR assay**) was utilized.
- BioRad QX200 Droplet Digital PCR system was used to detect and quantify viral RNA (cDNA) in each sample using SARS-CoV-2 N1 and N2 primer/probe sets published by US CDC.
- The 20 µL reaction mix consist of: 5 µL 2× one-step RT-ddPCR Supermix (Bio-Rad), 2 µL reverse transcriptase, 1 µL 300 mM DTT, 2 µL primers/probes, 5 µL RNAse free water and 5 µL RNA template.

Primer/ Probe	Primer/ Probe Sequence (5'-3')	Amplicon Size	Target gene	Final Conc.
N1-F	GACCCCAAAATCAGCGAAAT	72 bp	Nucleocapsid (N)	500 nM
N1-R	TCTGGTTACTGCCAGTTGAATCTG			500 nM
N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1			125 nM
N2-F	TTACAAACATTGGCCGCAAA	67 bp		500 nM
N2-R	GCGCGACATTCCGAAGAA			500 nM
N2-P	FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1			125 nM

METHODOLOGY

- Quantification of SARS-CoV-2 viral RNA
- Each sample was run in at least two replicates. No template control (NTC) and positive control (plasmid containing complete nucleocapsid gene from SARS-CoV-2) were included in the PCR reactions.
- Droplets were generated using 70 µL of oil per cartridge well and subsequently, 40 µL of generated droplets were thermocycled to endpoint.
- Thermocycling conditions were optimized prior to application.
- Samples were then quantified using the BioRad Plate reader coupled with QuantaSoft analysis software.

Cycling Step	Temperature (°C)	Time	Number of Cycles
Reverse Transcription	50	60 minutes	1
Enzyme activation	95	10 minutes	1
Denaturation	94	30 seconds	40
Annealing/Extension	55	60 seconds	
Enzyme Deactivation	98	10 minutes	1
Droplet Stabilization	4	30 minutes	1
Hold (optional)	4	24 hours	1



METHODOLOGY

- **RT-ddPCR Data Analysis**
- Analysis for N1 and N2 target copies/uL was conducted via manual thresholding.
- Validity of droplet reading was based on the number of total droplets in a sample well. A sample well with **more than 7000 droplets** is considered to be valid.
- A positive sample is indicated by a reading of **at least 3 positive droplets.**
- The average RNA concentration per 20 uL reaction volume is then converted to copies/L of wastewater.

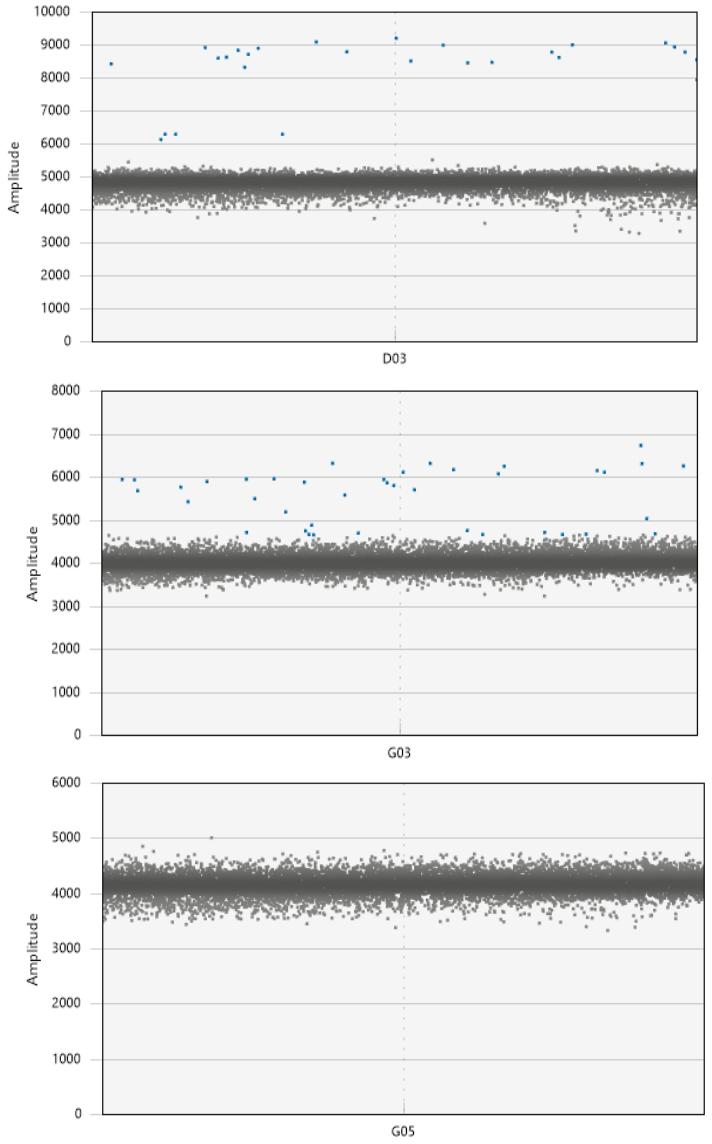


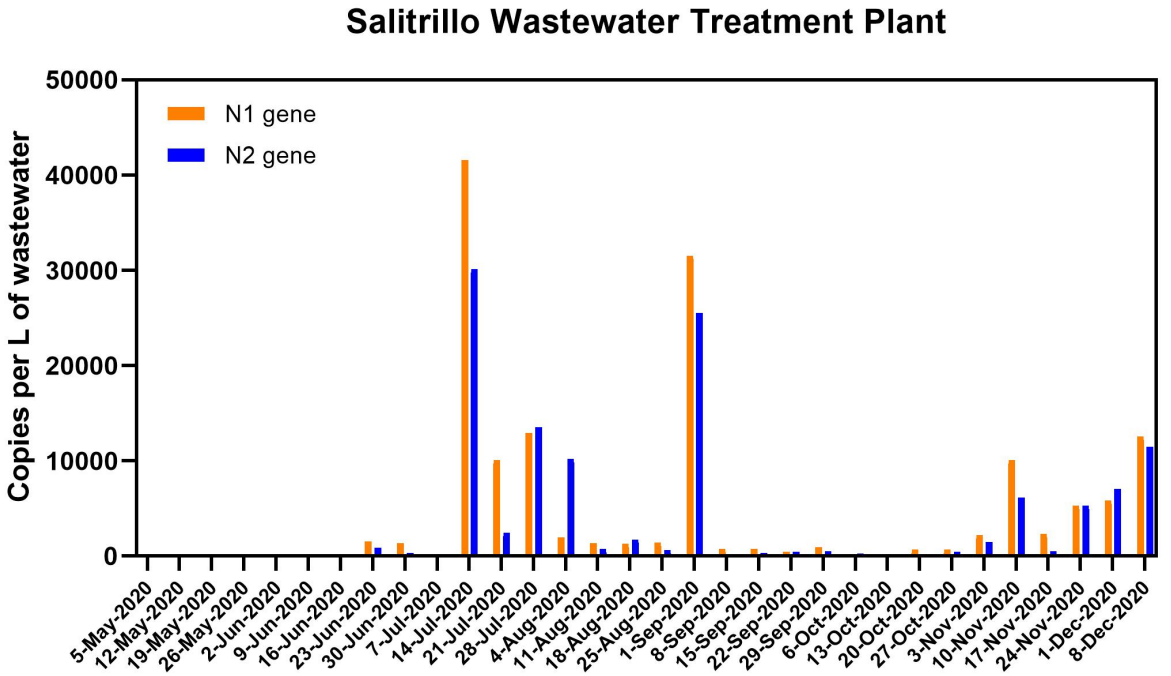
Figure 1. ddPCR detection panels. Panel D03, G03 and G05 shows a positive N1 sample, positive N2 sample and no template control (NTC). Positive droplets are shown in blue while negative droplets are highlighted in gray.

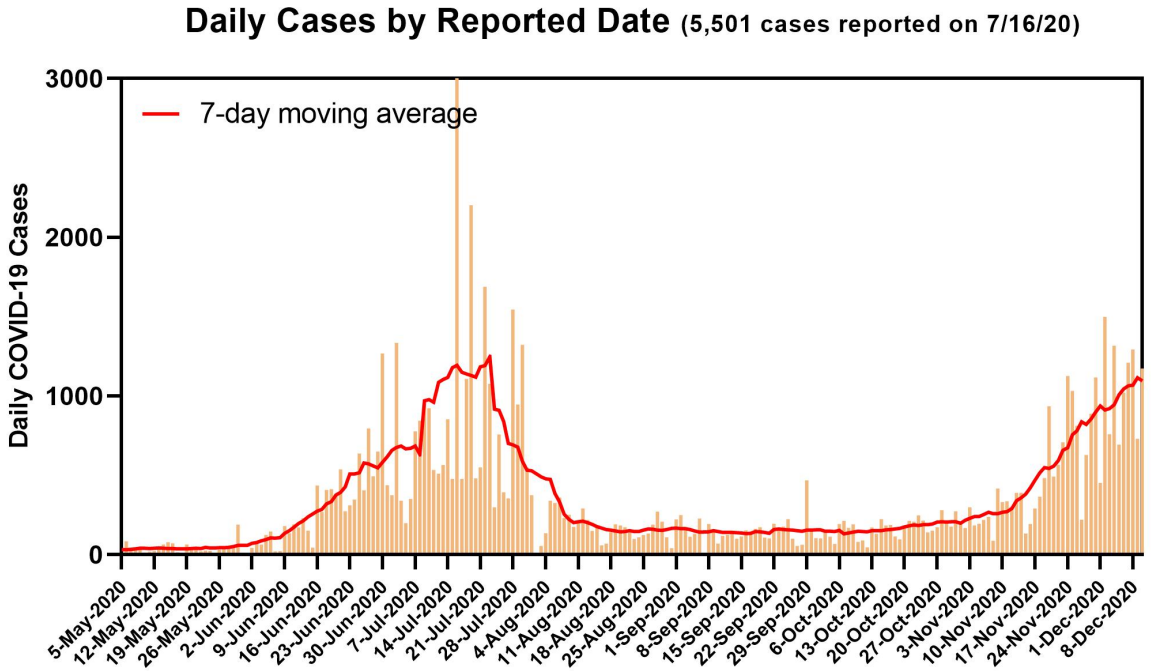
RESULTS

- Most samples collected starting June 2020 have tested **positive** for N1 and N2 gene targets.
- This allowed direct comparison of SARS-CoV-2 levels in different time periods where high and low measurements are anticipated.
- Comparison between viral RNA concentrations and number of reported clinical cases was also established.
- The current data correlation was conducted using Bexar County's daily case numbers recorded on the respective collection dates.

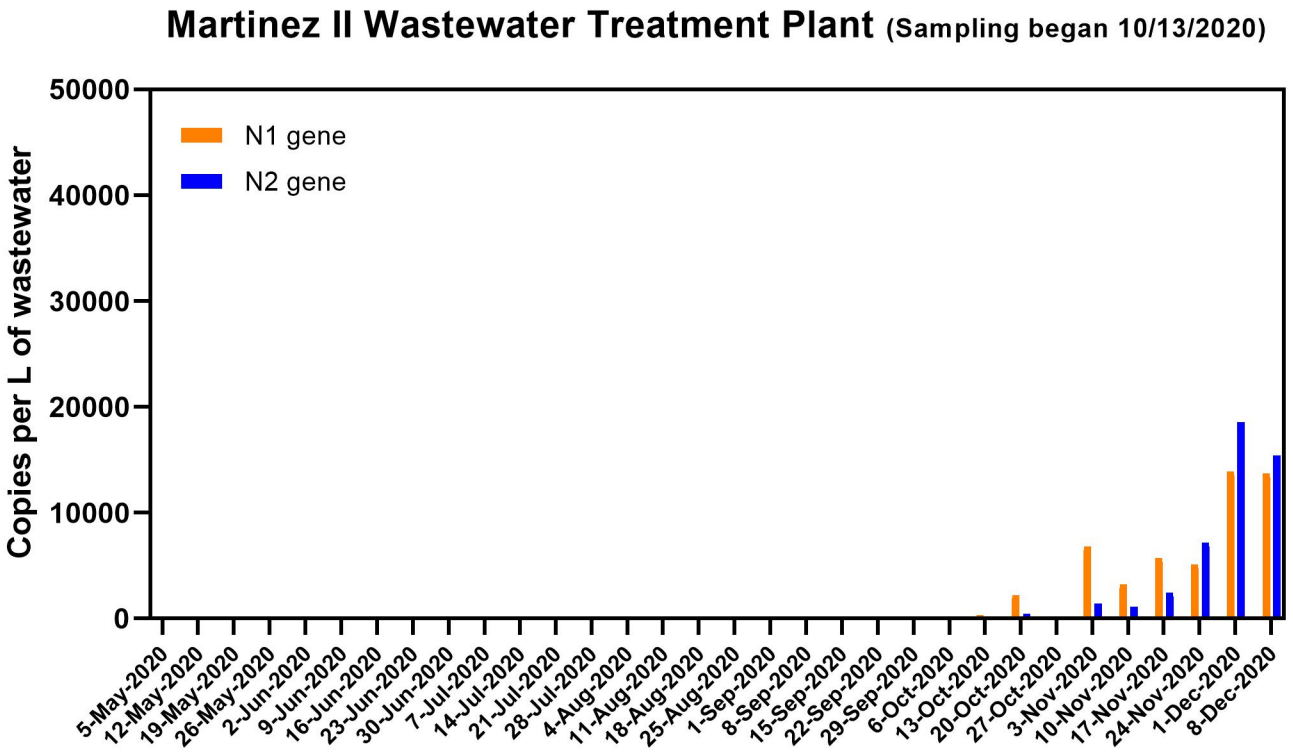
  Source: City of San Antonio

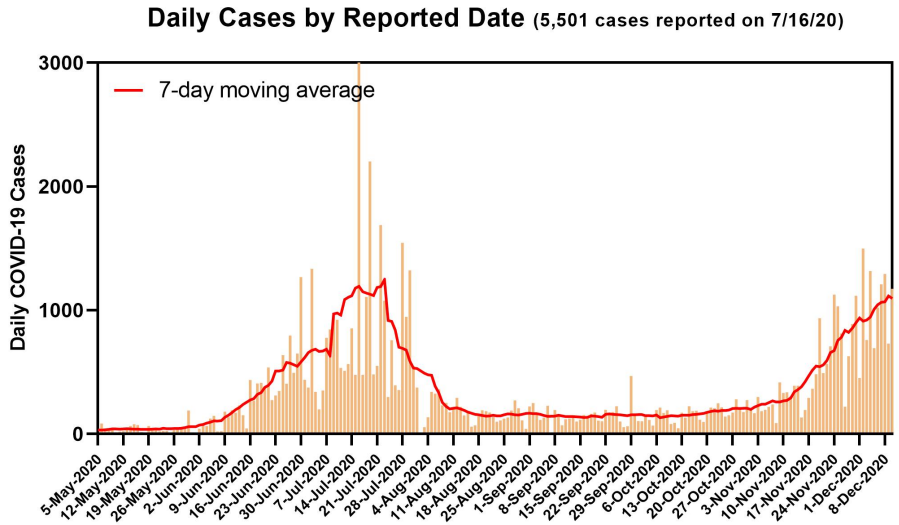
- On average, an increase in the concentration of viral RNA was observed which correlates well with an increase in the number of cases in the catchment area.





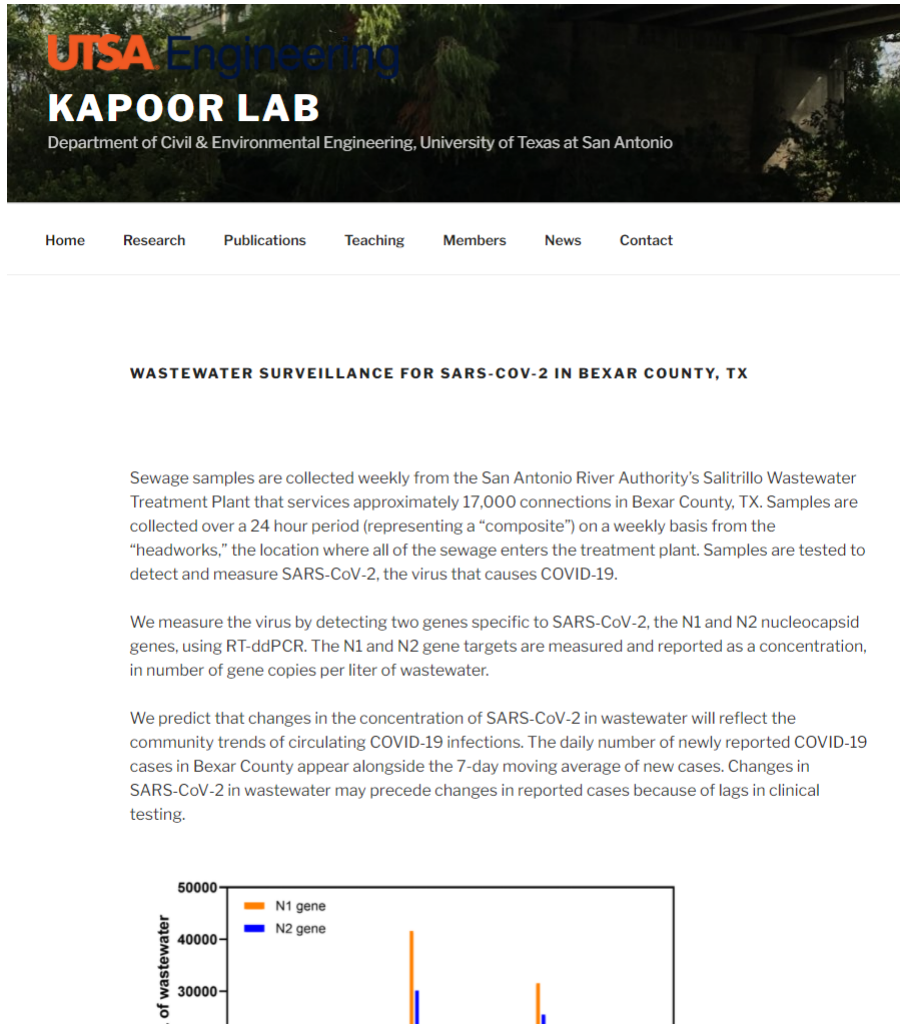
THE THANKSGIVING EFFECT !!





CONCLUSION

- The quantitative data on SARS-CoV-2 prevalence was reported out in viral RNA copies per volume of wastewater for the specific treatment service area.
- The average SARS-CoV-2 RNA concentrations were between 10^2 and 10^5 copies/L.
- The results showed a positive correlation with the number of reported clinical cases over the sampling time period.
- Viral RNA can be successfully concentrated and detected from raw influent wastewater using the RT-ddPCR method.
- For application of WBE of SARS-CoV-2 prevalence, further methodological validation is needed covering aspects from effective viral extraction and concentration through to data interpretation.
- Effective implementation of our data will require collaborations with local public health agencies, private utilities and affected communities in order to balance privacy concerns and public health management.

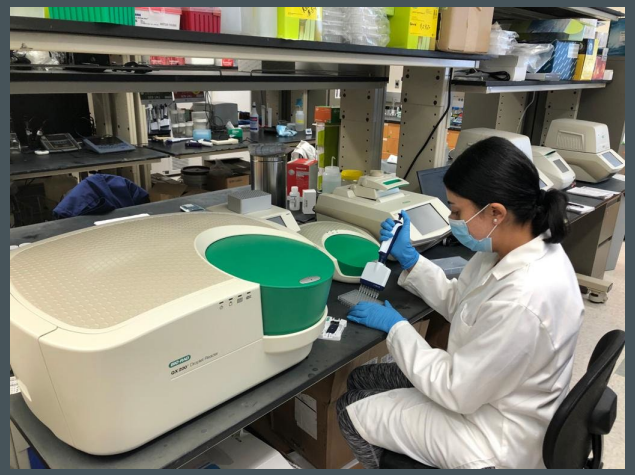


Link to Dashboard:
https://engineering.utsa.edu/vkapoor/research/wastewater-covid/

CURRENT WORK

- Normalization of data using bovine coronavirus vaccine
- Sampling extended to Martinez WWTP
- Establishing a robust filtration and RNA concentration method
- Automatic thresholding using a new ddPCR data analysis tool.
- Testing of effluent samples

ACKNOWLEDGEMENTS



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