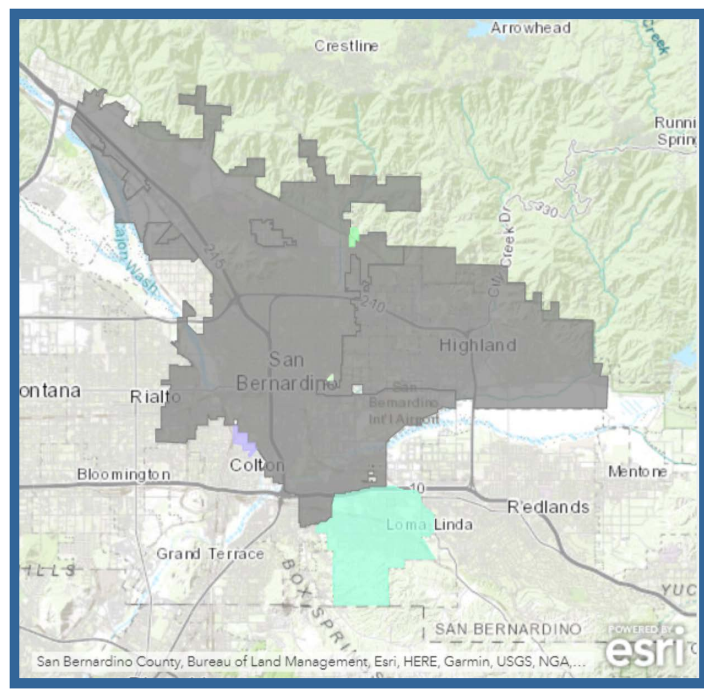

Developed and Validated: A Wastewater Based Epidemiology sampling method at Loma Linda University to support the WBE surveillance tool for the COVID-19 pandemic

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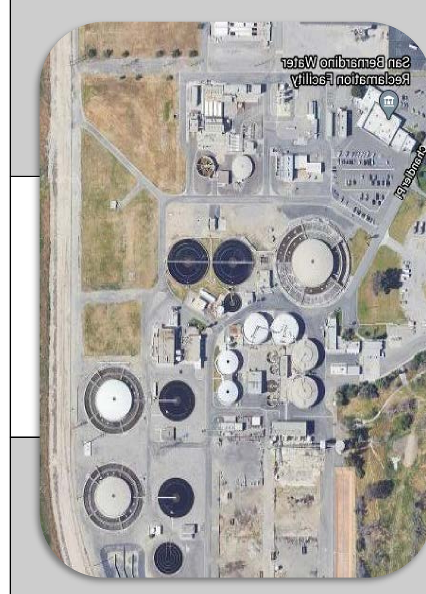
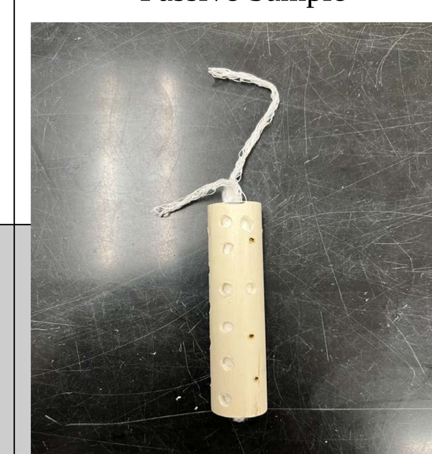
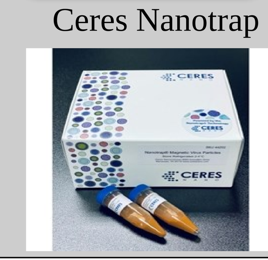
Introduction:

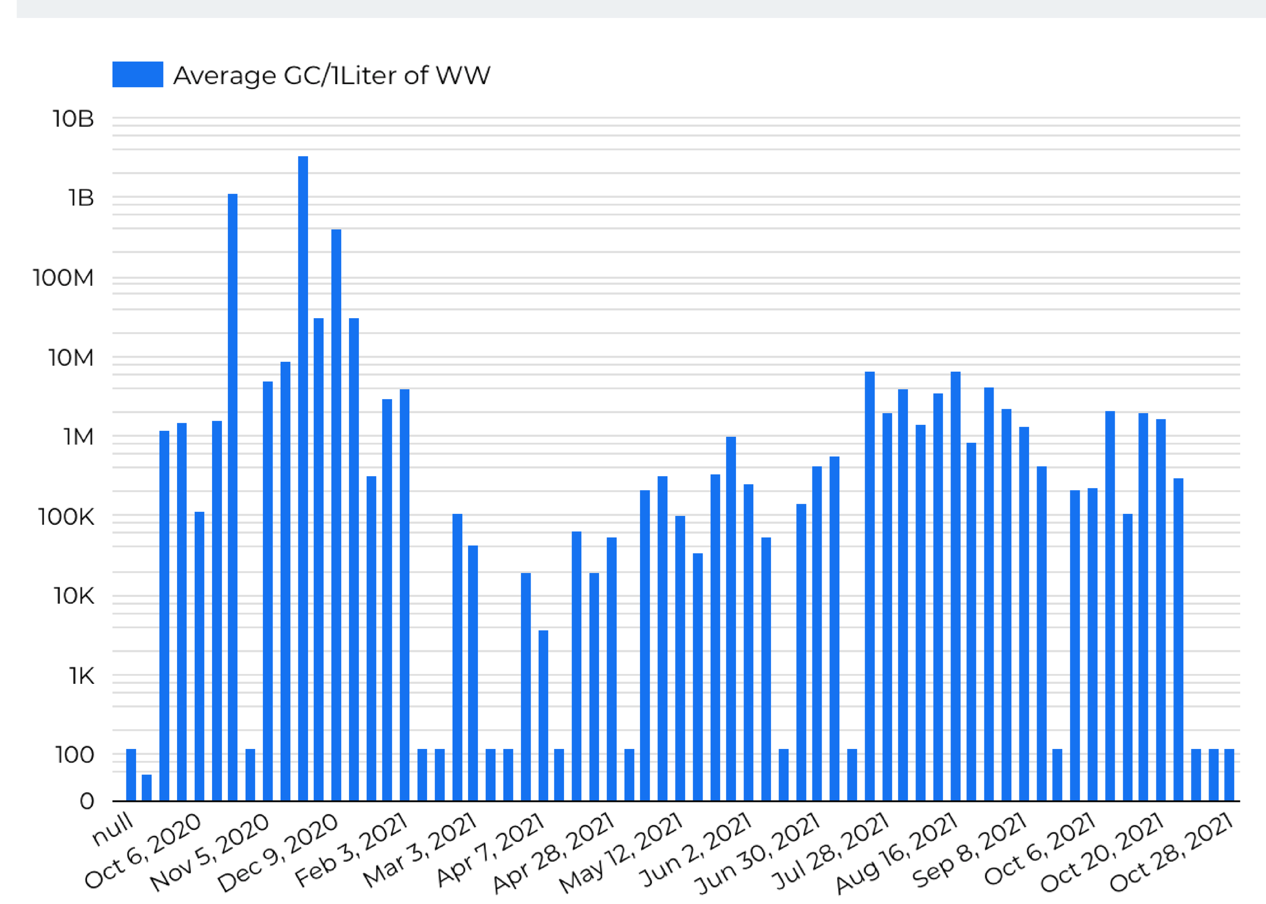
Wastewater Based Epidemiology (WBE) can be used to examine wastewater for the SARS-CoV-2 signal from defined sewage systems. The WBE surveillance tool is used for the early detection of biological weapons, infectious disease outbreaks, and indications of vaccination coverage. According to the CDC WBE is considered a supplemental surveillance method to detect and monitor for the presence of SARS-CoV-2. Before COVID-19 wastewater monitoring was commonly used as a surveillance tool to detect poliovirus vaccine coverage. Wastewater can be monitored with the sensitivity to detect less than 10 gene copies of poliovirus per liter of wastewater. The WBE system has been shown to give a 4 to10 day early warning on impending COVID-19 outbreaks. The laboratory techniques have constantly been evolving as researcher have used various concentration, sampling, extraction methods to find which method is most effective in reporting the presence of the pathogen in wastewater. The challenges WBE faces when monitoring the presence of SARS-CoV-2 is the uncertainty of the genetic sequencing of variants found within the wastewater samples, the use of high sensitivity laboratory analysis methods to detect low number of pathogens in a sample and presenting the data in a timely and efficient way to indicate the sewer signal to COVID-19 case rates. Loma Linda University's WBE laboratory has collected samples for one year from three locations on campus and compared them to a baseline composite sample from San Bernardino County's (SBC) wastewater treatment plant as seen on the Live Dashboard. This study is investigating the cost-effective passive sampling method to determine whether it was effective at detecting SARS-CoV-2 with the same detection rate and sensitivity as the standard grab sampling method.

Methods:

The passive sampler used a sterile packaged sanitary cloth placed in a PVC pipe with holes drilled into the sides for water passage. These samples were left in the sewage flow for 24 hours at three campus locations and then approximately 50ml of liquid wastewater was eluted from the cloth. For comparison, a grab sample method was also used at a discrete time point when the passive sampler was first placed at the campus locations. The grab sample used a peristaltic pump to collect 1 Liter of wastewater from clean out ports next to campus buildings. A comparable composite sample was collected weekly from the SBC. A weekly sample was collected for six months and evaluated using qPCR. The team first concentrated wastewater samples, then extracted RNA before processing with qPCR. A RT-qPCR analysis was followed using the CDC standard RUO protocol and standard curves for concentration estimates with the RNA targets of N1 and N2. All filter samples were processed with negative filter controls and duplicate technical replicates (5ul) for each N target on the RT-qPCR. Results are given in the number of gene copies per liter of wastewater (GC/L). The final RT-qPCR data points were analyzed and visualized using SPSS 28 to compare detection sensitivity and quantification cycle values using paired t-tests for significance. All values lower than the limit of quantification (LLOQ) were initially coded as non-detects, then substituted with a number half the value of the LOQ to allow statistical comparative analysis without missing values. The SARS-CoV-2 that were detected using the passive sample method was examined by comparing the quantification cycle (cq) values, the number of positive detections above the LLOQ, and how close they were to the cq values from the SB composite samples collected downstream at the wastewater treatment plant.

Composite Sample: San Bernardino Wastewater Partnership with SBC WWTP	**Sampling Method:** Grab Sample	**Concentration Method:** HA Filters	**Extraction**	**qPCR**
			Beat Beating and centrifugation 	Reverse Transcriptase quantitative PCR for N1 and N2.
	Passive Sample 	Millipore HA Filters 	Extraction Kits 	
		Ceres Nanotrap 	Controls : BRSV & PMMOV Bovine Respiratory Syncytial Virus and Pepper Mild Mottle Virus 	

Bar chart with Gene Copies per Liter in San Bernardino wastewater shown with log transformed values.



Bar chart with Gene Copies per Liter in another wastewater system that our lab is monitoring.

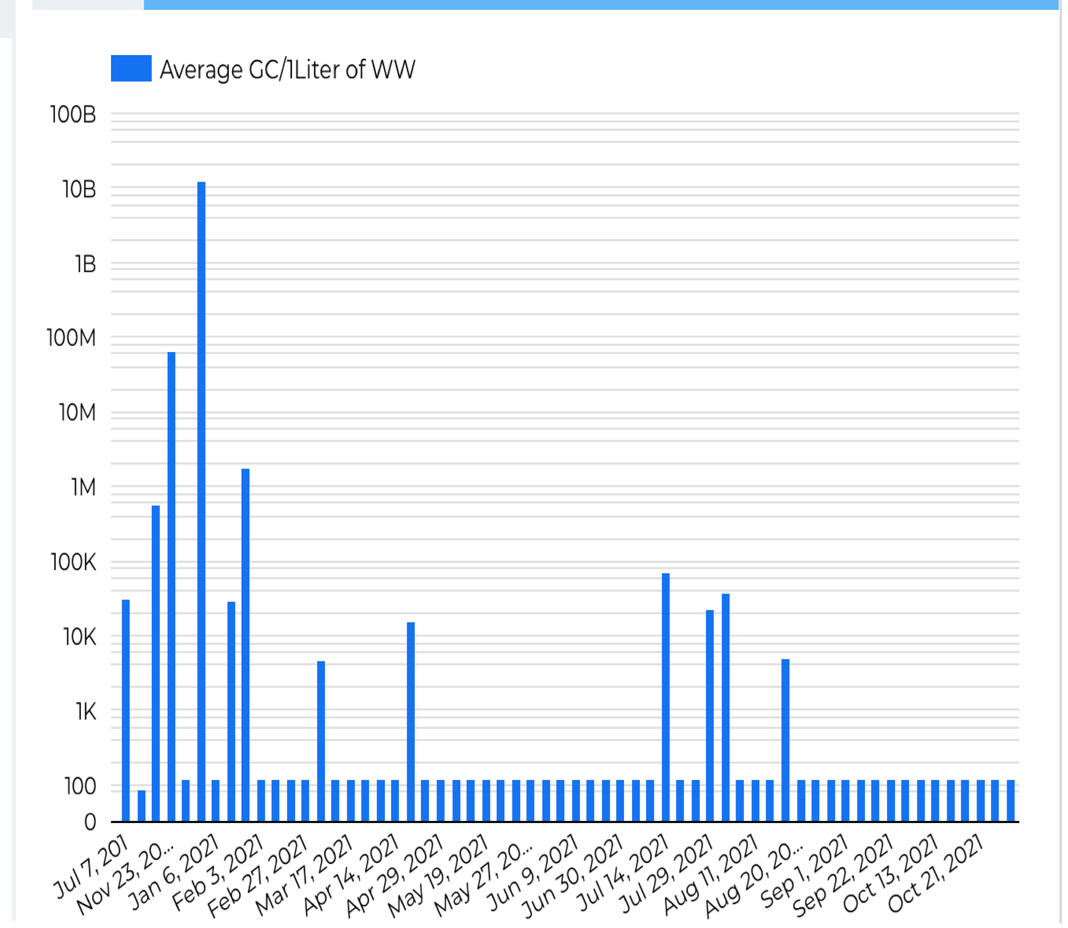


Table 1. *Sample Descriptive results comparing two types of sampling methods.*

Variable	Mean	Std. Dev.	Variance	df
Passive Sample	1.53	0.699	0.488	26
Grab Sample	1.89	0.858	0.737	26

**Log gene copies per liter.*

Table 2. *Paired sample correlations.*

	N	Correlation	Sig.
Passive & Grab	27	0.139	0.089

** Correlation is significant at the 0.05 level (2-tailed)*

SARS-CoV-2 RNA Concentration ($\log_{10}$ genome copies $\cdot$ L^{-1}) – N1 Assay

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Month	April			May				June				July			
Passive	ND	ND	ND	ND	2.9	ND	ND	ND	ND	3	ND	ND	ND	2.9	2
Grab	2.5	ND	3.1	ND	3.2	2.1	ND	ND	ND	2.1	ND	2.9	3.7	ND	2.3

Week	16	17	18	19	20	21	22	23	24	25	26
Month	August					September		October			
Passive	3	ND	ND	2	ND	ND	ND	2.7	ND	ND	ND
Grab	3	2.1	2.2	2.3	ND	ND	ND	1	ND	ND	ND

Low High

ND: Not Detected

Results:

The paired comparisons verified that the passive sampling method was reasonably sensitive at detecting SARS-CoV-2 in wastewater. Overall, the two sampling methods did not produce a significant difference when using paired comparisons. Seven- months of sampling were examined and 26 days were evaluated, and results showed that 19 passive samples were non-detects and 13 grab samples were non-detects. There were six occurrences where the grab sample had detections that the passive sampler did not. The paired t-test did not show a significant difference between the two methods.

Conclusion:

The project was successful in monitoring the San Bernardino wastewater treatment plant from October 2020 through October 2021 obtaining similar values to that of previous commercial laboratories that processed WBE data. Moving forward the LLU WBE team in partnership with SB county and community stakeholders will apply these validated surveillance protocols for disadvantaged communities. This method of surveillance is most useful in detecting the occurrence of the virus in small populations alongside active testing regimes. The WBE research team found low levels of viruses from grab samples of sewage from confined populations with less than 2 active COVID-19 cases. When the COVID-19 cases were resolved the SARS-CoV-2 signal was no longer present in the wastewater. WBE shows utility for monitoring all phases of the pandemic including the exponential infection rate increase and low vaccine coverage rates. The LLU campus benefits from WBE by the early detection of a COVID-19 outbreak and routine monitoring of confined populations. In the early response, LLU quickly developed a WBE response committee to decide on communication strategies for the results that were found with this method. Further research will focus on optimizing the deployment of passive samplers to various SBC locations to understand the presence of SARS-CoV-2 variants.

References

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1. Sinclair, Ryan G., Christopher Y. Choi, Mark R. Riley, and Charles P. Gerba. "Pathogen Surveillance through Monitoring of Sewer Systems." *Advances in Applied Microbiology* 65 (2008): 249–69. https://doi.org/10.1016/S0065-2164(08)00609-6.

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