SARS-CoV-2
WASTEWATER TESTING REPORT

August 13, 2021
Account: Town of Big Sky
Result Summary: Influent Sample - Detected

Sample 1: WWTP Influent

Sample ID	Estimated Genomes per Liter	Call	
Influent 1 N1	14089	Positive	**Average N1:**
Influent 2 N1	13617	Positive	13853
Influent 1 N2	7382	Positive	**Average N2:**
Influent 2 N2	11945	Positive	9664

Sample Descriptions

Sample 1 – A composite sample of wastewater inflow to the wastewater treatment plant was captured over 24 hours on 8/11/2021. Two 40mL sub-samples were taken from the composite and processed in parallel, referred to as Influent 1 and Influent 2 in the table above.

Test Information

Each sample was tested for the presence of two nucleocapsid genes present in SARS-CoV2, N1 and N2. Signal from the test was plotted against a standard curve made of known copy numbers of N1 and N2 to quantify genomes per liter. Samples with signal that is lower than the lowest dilution on the standard curve are considered to be below the limit of detection (L.O.D.) and labeled "BELOW L.O.D." Note that they are still considered positive.

Interpretation

Signal for the presence of virus was observed in Influent samples (Ct < 40 using CDC qPCR assay). Based on our experience with wastewater testing, this is strong evidence of low levels of virus in wastewater. Measured concentration of virus in wastewater is lower than the previous week.

Relevant text from CDC guidelines:

> *"…a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct)."*
>
> *"When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive."*

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Testing Details

Testing for the presence and abundance of the SARS-CoV2 genome in the above samples was performed using a kit designed by the US Centers for Disease Control and Prevention (CDC 2019-Novel Coronavirus (2019-nCoV), Real-Time RT-PCR Diagnostic Panel). The test was used here to determine whether a detectible amount of viral genome was present. Each of the above samples were split and processed as two replicates. RNA was isolated from inactivated/concentrated replicates, reverse-transcribed to DNA and used as template in quantitative PCR reactions as per kit instructions. Duplicate CDC-designed tests were performed on each replicate to detect two distinct locations on the SARS-CoV2 genome (N1 and N2). Genome numbers were quantified using a standard curve of known copy numbers of N1 and N2 generated using the same PCR assay.

Wastewater surveillance for SARS-CoV2 is novel and a developing technology, so results must be taken with consideration. Virus levels may fluctuate due to flow rates, flow volumes, and other factors. Absence of viral signal may not mean complete absence of virus in the community, although presence of viral signal certainly means presence of virus in the community at the time of sampling. Comparisons among sampling sites (e.g. between municipalities) is not recommended. Archer Biologicals, LLC is not responsible for misinterpretation of the presented data. For more information, visit our website: www.archerbiologicals.com/wastewatersurveillance

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