

August 3, 2020*

West Yellowstone Wastewater Testing Results

Result Summary: West Yellowstone Sample was positive

Sample Description:

- 1) A composite sample of wastewater (1.0 L total) inflow to the West Yellowstone treatment plant was captured on 7/22/2020 using an auto-sampler over the previous 24-hour period. Referred to below as “Inflow” samples in **Table 1**.

Testing Information and Raw Data:

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). *Importantly, this test kit was originally designed to detect the virus in human samples and NOT wastewater or other kinds of environmental samples.* The test was used here to determine whether a detectable amount of virus was present. Results need to be interpreted with caution, as described below.

Each of the above samples were split and processed as three replicates. Two tests were performed on each replicate and two independent locations on the SARS-CoV2 genome were targeted (N1 and N2). RNA was isolated from inactivated/concentrated samples, reverse-transcribed to DNA and used as template in quantitative PCR reactions as per kit instructions. Results were recorded as cycle threshold (Ct) numbers based on test interpretation guidelines described by the CDC. A standard curve was generated using a pre-made virus target and used to calculate the number of genomes in each sample.

*NOTE: These data correct a previously published version of this document. Previous data did not include a correction factor to reflect a change in protocol. The interpretation of “Positive” has not changed. The data have also been reorganized to more intuitively reflect replicate samples.

Results were as follows:

West Yellowstone Sample ID	Replicate ID	Target	Ct	Potential Genomes per liter
Inflow_1	N1.1	N1	33.3171	284230
Inflow_1	N1.2	N1	32.9805	357034
Inflow_1	N2.1	N2	34.4728	129903
Inflow_1	N2.2	N2	34.7395	108429
Inflow_2	N1.1	N1	33.5161	248380
Inflow_2	N1.2	N1	33.5934	235706
Inflow_2	N2.1	N2	35.0396	88480
Inflow_2	N2.2	N2	34.8173	102862
Inflow_3	N1.1	N1	33.4337	262640
Inflow_3	N1.2	N1	33.7008	219165
Inflow_3	N2.1	N2	34.8936	97680
Inflow_3	N2.2	N2	34.9103	96581

Interpretation:

Signal in all replicates was above our limit of detection. Based on our experience with wastewater testing, this is strong evidence of virus in wastewater. Levels of virus in this sample was increased from the previous week's sample (taken 7/15/2020).

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."