

June 18, 2020

## West Yellowstone Wastewater Testing Results

### Result Summary: West Yellowstone Sample was negative

#### Sample Description:

- 1) A composite sample of wastewater (1.0 L total) inflow to the West Yellowstone treatment plant was captured on 6/17/2020 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.

Results were as follows:

West Yellowstone Sample ID	Replicate ID	Target	Ct	Potential Genomes per liter
Inflow_1	Inflow_1.1	N1	NA	NA
Inflow_1	Inflow_1.1	N2	43.1914	104
Inflow_1	Inflow_1.2	N1	37.2242	1318
Inflow_1	Inflow_1.2	N2	44.7575	33
Inflow_2	Inflow_2.1	N1	40.0783	127
Inflow_2	Inflow_2.1	N2	42.6716	152
Inflow_2	Inflow_2.2	N1	NA	NA
Inflow_2	Inflow_2.2	N2	NA	NA
Inflow_3	Inflow_3.1	N1	40.0436	130
Inflow_3	Inflow_3.1	N2	43.3523	93
Inflow_3	Inflow_3.2	N1	38.7388	380
Inflow_3	Inflow_3.2	N2	NA	NA

#### Interpretation:

Signal for the presence of virus was observed but was either very low (>40 Ct) or not consistent between replicate samples for either the N1 or N2 targets. Signal in only two of the replicates was within the CDC recommended guidelines for positivity (i.e. <40 Ct). Based on our experience with wastewater testing, there is insufficient evidence that the virus was present in this sample. That said, it is concerning that signal, albeit low, was detected in 8 of the 12, which could indicate that the virus is present but at a low abundance. This signal deserves attention and will be re-examined next 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.”