June 26, 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 6/24/2020 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 detectible 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 Yellostone Sample ID	**Replicate ID**	**Target**	**Ct**	**Potential Genomes per liter**
Inflow_1	N1pve	Unknown	38.4217	110.6270136
Inflow_1	N2pve	Unknown	40.2189	39.01573573
Inflow_1	N1pve	Unknown	38.3874	113.9096473
Inflow_1	N2pve	Unknown	NA	NA
Inflow_2	N1pve	Unknown	NA	NA
Inflow_2	N2pve	Unknown	40.0059	47.18818844
Inflow_2	N1pve	Unknown	38.3937	113.2994964
Inflow_2	N2pve	Unknown	42.0836	7.382071798
Inflow_3	N1pve	Unknown	NA	NA
Inflow_3	N2pve	Unknown	41.2752	15.19307976
Inflow_3	N1pve	Unknown	39.0006	67.53482687
Inflow_3	N2pve	Unknown	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 4 of 12 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, this is the second week in a row that signal was detected, albeit low, in at least 8 of the 12 replicates, 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."