June 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 6/2/2020 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) if observed before 40 cycles 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, if a signal (<40 Ct) was observed.

Results were as follows:

Table 1
West Yellowstone

Sample ID	Replicate ID	Target	Ct	Potential Genomes per liter
Inflow_1	Inflow_1.1	N1	33.3773	20030
Inflow_1	Inflow_1.1	N2	34.8549	26265
Inflow_1	Inflow_1.2	N1	33.5541	17543
Inflow_1	Inflow_1.2	N2	35.2047	20523
Inflow_2	Inflow_2.1	N1	33.9159	13376
Inflow_2	Inflow_2.1	N2	35.6616	14870
Inflow_2	Inflow_2.2	N1	33.6631	16167
Inflow_2	Inflow_2.2	N2	36.4523	8514
Inflow_3	Inflow_3.1	N1	33.2936	21327
Inflow_3	Inflow_3.1	N2	35.3432	18614
Inflow_3	Inflow_3.2	N1	33.096	24732
Inflow_3	Inflow_3.2	N2	35.4383	17406

Interpretation:

Per CDC guidelines, all samples from West Yellowstone produced signal well below the 40 Ct cut-off. *These results are consistent with the presence of SARS-CoV2 in the wastewater sample*. Estimated numbers of virus in this sample were greater than the previous sample (see figure below).

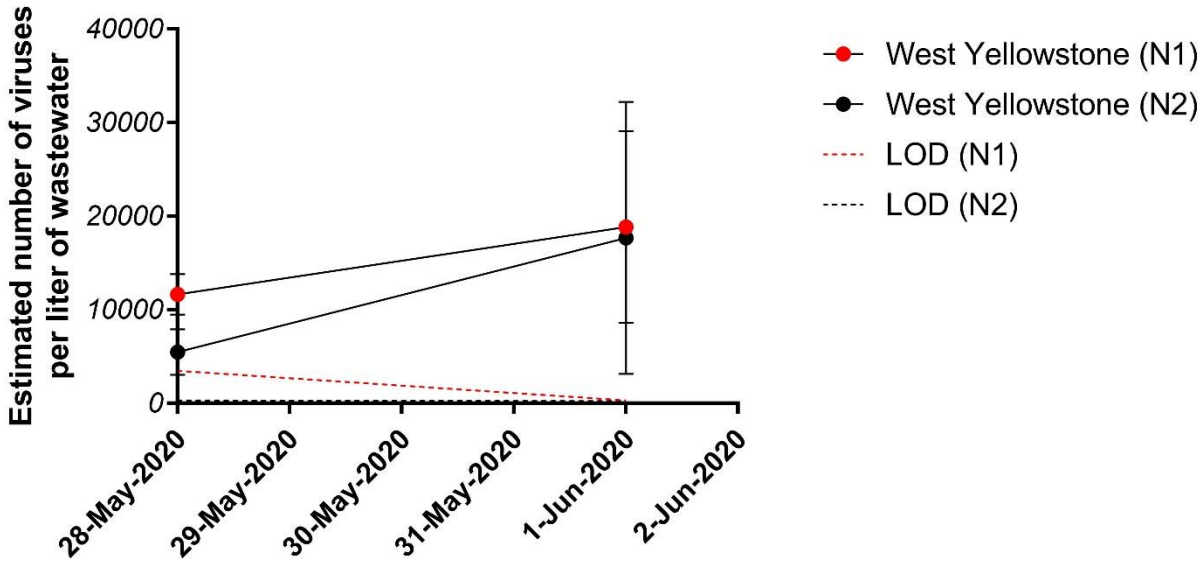

Figure. Estimated number of SARS-CoV2 in wastewater samples from the town of West Yellowstone, MT. N1 = nucleocapsid gene target 1, N2 = nucleocapsid gene target 2, LOD = limit of detection.

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."