

May 30, 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) from a main pump station in the town of West Yellowstone was captured on 5/29/2020 using an auto-sampler over the previous 24-hour period. Referred to below as "PumpStation" 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) 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:

<b>Sample ID</b>	<b>Replicate ID</b>	<b>Target</b>	<b>Ct</b>	<b>Potential Genomes per liter</b>
PumpStation_1	PumpStation_1.1	N1	33.2275	9797
PumpStation_1	PumpStation_1.1	N2	35.2586	4639
PumpStation_1	PumpStation_1.2	N1	32.9946	11699
PumpStation_1	PumpStation_1.2	N2	35.0615	5508
PumpStation_2	PumpStation_2.1	N1	33.39	8657
PumpStation_2	PumpStation_2.1	N2	34.8542	6599
PumpStation_2	PumpStation_2.2	N1	32.5585	16307
PumpStation_2	PumpStation_2.2	N2	34.8555	6591
PumpStation_3	PumpStation_3.1	N1	32.8254	13308
PumpStation_3	PumpStation_3.1	N2	35.0221	5701
PumpStation_3	PumpStation_3.2	N1	33.1744	10202

PumpStation_3	PumpStation_3.2	N2	35.4889	3796
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Interpretation:

All samples from West Yellowstone produced signal well below the 40 Ct cut-off set by the CDC. *These results are consistent with the presence of SARS-CoV2 in the wastewater sample.*

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.”