

June 18, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky sample was positive

Sample Description:

A composite sample of wastewater (1.5 L total) inflow to the Big Sky treatment plant was captured on 6/16/2020 using an auto-sampler over the previous 24-hour period. Referred to below as “Inflow” samples.

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. All Ct numbers above (greater than) 40 cycles were highlighted in the table. Based on test interpretation guidelines described by the CDC (see below) Ct numbers greater than 40 should not be considered positive.

Results were as follows:

Big Sky				Potential Genomes per liter
Sample ID	Replicate ID	Target	Ct	
Inflow_1	Inflow_1.1	N1	36.9193	2544
Inflow_1	Inflow_1.1	N2	39.1218	1441
Inflow_1	Inflow_1.2	N1	36.4920	3499
Inflow_1	Inflow_1.2	N2	40.0855	703
Inflow_2	Inflow_2.1	N1	36.9985	2398
Inflow_2	Inflow_2.1	N2	39.3150	1248
Inflow_2	Inflow_2.2	N1	38.3677	863
Inflow_2	Inflow_2.2	N2	39.7752	886
Inflow_3	Inflow_3.1	N1	38.3436	879
Inflow_3	Inflow_3.1	N2	40.7704	422
Inflow_3	Inflow_3.2	N1	37.9586	1171
Inflow_3	Inflow_3.2	N2	40.6761	453

Interpretation:

Virus signal increased from last week for both N1 and N2 targets showing Ct numbers in 9 of 12 replicates ≤ 40 . Signal for the N2 target was right at the detection limit but unlike the previous week averaged < 40 (average=39.96). Based on our experience with testing environmental and wastewater samples and results from previous weeks of testing, we feel these results are consistent with a positive result.

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.”