

June 25, 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 6/23/2020 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	33.9485	9468
Inflow_1	Inflow_1.1	N2	36.1613	23931
Inflow_1	Inflow_1.2	N1	34.275	7305
Inflow_1	Inflow_1.2	N2	36.0171	26051
Inflow_2	Inflow_2.1	N1	34.5034	6093
Inflow_2	Inflow_2.1	N2	36.0544	25485
Inflow_2	Inflow_2.2	N1	34.2023	7739
Inflow_2	Inflow_2.2	N2	37.6058	10226
Inflow_3	Inflow_3.1	N1	34.1884	7825
Inflow_3	Inflow_3.1	N2	36.0343	25789
Inflow_3	Inflow_3.2	N1	33.7296	11265
Inflow_3	Inflow_3.2	N2	36.8311	16134

Interpretation:

Virus signal increased significantly from last week for both N1 and N2 targets, showing Ct numbers in all replicates well below 40. These results are entirely consistent with a positive result. The steep upward trend in virus levels is concerning as only a single case has been confirmed in Big Sky to date. Since significantly more virus was present this week versus last week, it seems reasonable that more cases will be confirmed.

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.”