

October 2, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky Inflow Sample was positive; Irrigation Sample was negative

Sample Description:

- 1) A composite sample of wastewater inflow (1.0 L total) to the Big Sky treatment plant was captured on 9/30/2020 using an auto-sampler over the previous 24-hour period. Referred to below as “Inflow” samples in **Table 1**.
- 2) A single-catch sample (1.0 L total) of water used for irrigation of the Big Sky Golf Course was captured on 9/30/2020. Referred to below as “Irrigation” samples in **Table 1** below.

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 two 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.

Note: Previous test results included three replicate samples. These results also use a correction factor of 2.31 cycles (Cts) to adapt a new method to improve sensitivity.

Results were as follows:

Big Sky Sample ID	Replicate ID	Target	Ct	Potential Genomes per liter
Inflow_1	N1.1	N1	36.5249	1724
Inflow_1	N1.2	N1	35.4462	4207
Inflow_1	N2.1	N2	38.0096	505
Inflow_1	N2.2	N2	37.7121	646
Inflow_2	N1.1	N1	35.731	3324
Inflow_2	N1.2	N1	35.4116	4329
Inflow_2	N2.1	N2	37.4619	794
Inflow_2	N2.2	N2	37.301	907
Irrigation_1	N1.1	N1	Undetermined	0
Irrigation_1	N1.2	N1	Undetermined	0
Irrigation_1	N2.1	N2	Undetermined	0
Irrigation_1	N2.2	N2	Undetermined	0
Irrigation_2	N1.1	N1	Undetermined	0
Irrigation_2	N1.2	N1	Undetermined	0
Irrigation_2	N2.1	N2	Undetermined	0
Irrigation_2	N2.2	N2	Undetermined	0

Interpretation:

Signal in all replicates of the inflow sample was above our limit of detection. Based on our experience with wastewater testing, this is evidence of virus in wastewater. Levels of virus in the inflow were lower relative to the previous week's sample (taken 9/23/2020).

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." In these reports, Cts reported below 42.31 are considered positive, as virus was detectable using more sensitive methods.