

August 8, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky Wastewater Sample was positive; Irrigation Pond sample was negative

Sample Description:

- 1) A composite sample of wastewater (1.0 L total) inflow to the Big Sky treatment plant was captured on 8/5/2020 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 8/5/2020. Referred to below as “Irrigation” samples in Table 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 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) 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.

Results were as follows:

Big Sky Inflow Sample ID	Replicate ID	Target	Ct	Potential Genomes per liter
Inflow_1	N1.1	N1	33.3497	515093
Inflow_1	N1.2	N1	33.3685	507367
Inflow_1	N2.1	N2	33.8257	826813
Inflow_1	N2.2	N2	33.7739	860058
Inflow_2	N1.1	N1	33.7151	383990
Inflow_2	N1.2	N1	33.6525	403808
Inflow_2	N2.1	N2	33.8631	803611
Inflow_2	N2.2	N2	33.8148	833700
Inflow_3	N1.1	N1	33.5621	434245
Inflow_3	N1.2	N1	33.5138	451436
Inflow_3	N2.1	N2	33.9076	776852
Inflow_3	N2.2	N2	33.8029	841285

Big Sky Irrigation Pond				Potential
Sample ID	Replicate ID	Target	Ct	Genomes per liter
Irrig_1	N1.1	N1	Undetermined	0
Irrig_1	N1.2	N1	Undetermined	0
Irrig_1	N2.1	N2	Undetermined	0
Irrig_1	N2.2	N2	Undetermined	0
Irrig_2	N1.1	N1	Undetermined	0
Irrig_2	N1.2	N1	Undetermined	0
Irrig_2	N2.1	N2	Undetermined	0
Irrig_2	N2.2	N2	Undetermined	0
Irrig_3	N1.1	N1	Undetermined	0
Irrig_3	N1.2	N1	Undetermined	0
Irrig_3	N2.1	N2	Undetermined	0
Irrig_3	N2.2	N2	Undetermined	0

Interpretation:

Signal in all replicates was above our limit of detection in the Inflow samples. Based on our experience with wastewater testing, this is strong evidence of virus in wastewater. Levels of virus in this sample was higher than the previous week's sample (taken 7/29/2020). Virus was undetectable in the irrigation pond 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."