

May 30, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky samples were negative

Sample Description:

- 1) A single-catch sample (1.0 L total) of water used for irrigation of the Big Sky Golf Course was captured on 5/28/2020. Referred to below as "Irrigation" samples in **Table 1**.
- 2) A composite sample of wastewater (1.0 L total) inflow to the Big Sky treatment plant was captured on 5/28/2020 using an auto-sampler over the previous 24-hour period. Referred to below as "Inflow" 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:

Table 1				Potential Genomes per liter
Big Sky				
Sample ID	Replicate ID	Target	Ct	
Inflow_1	Inflow_1.1	N1	Undetermined	
Inflow_1	Inflow_1.1	N2	Undetermined	
Inflow_1	Inflow_1.2	N1	Undetermined	
Inflow_1	Inflow_1.2	N2	Undetermined	
Inflow_2	Inflow_2.1	N1	Undetermined	
Inflow_2	Inflow_2.1	N2	Undetermined	
Inflow_2	Inflow_2.2	N1	Undetermined	
Inflow_2	Inflow_2.2	N2	Undetermined	
Inflow_3	Inflow_3.1	N1	Undetermined	
Inflow_3	Inflow_3.1	N2	Undetermined	

Inflow_3	Inflow_3.2	N1	Undetermined	NA
Inflow_3	Inflow_3.2	N2	Undetermined	NA
Irrigation_1	Irrigation_1.1	N1	Undetermined	NA
Irrigation_1	Irrigation_1.1	N2	Undetermined	NA
Irrigation_1	Irrigation_1.2	N1	Undetermined	NA
Irrigation_1	Irrigation_1.2	N2	Undetermined	NA
Irrigation_2	Irrigation_2.1	N1	Undetermined	NA
Irrigation_2	Irrigation_2.1	N2	Undetermined	NA
Irrigation_2	Irrigation_2.2	N1	Undetermined	NA
Irrigation_2	Irrigation_2.2	N2	Undetermined	NA
Irrigation_3	Irrigation_3.1	N1	Undetermined	NA
Irrigation_3	Irrigation_3.1	N2	Undetermined	NA
Irrigation_3	Irrigation_3.2	N1	Undetermined	NA

Interpretation:

Per CDC guidelines, no sample should be considered positive unless both genome targets are detected (see below). No samples from Big Sky met this criterion.

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.”