

June 5, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky 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 6/2/2020 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:

Big Sky				Potential Genomes per liter
Sample ID	Replicate ID	Target	Ct	
Inflow_1	Inflow_1.1	N1	NA	NA
Inflow_1	Inflow_1.1	N2	38.5855	547
Inflow_1	Inflow_1.2	N1	NA	NA
Inflow_1	Inflow_1.2	N2	39.013	385
Inflow_2	Inflow_2.1	N1	NA	NA
Inflow_2	Inflow_2.1	N2	38.7561	476
Inflow_2	Inflow_2.2	N1	NA	NA
Inflow_2	Inflow_2.2	N2	NA	NA
Inflow_3	Inflow_3.1	N1	NA	NA
Inflow_3	Inflow_3.1	N2	NA	NA
Inflow_3	Inflow_3.2	N1	NA	NA
Inflow_3	Inflow_3.2	N2	NA	NA

Interpretation:

Per CDC guidelines, the replicate samples from Big Sky produced either inconsistent signal or no signal at all ('NA' in Table above). Such results might be expected if there was a very low level of virus in the sample (scenario 1) OR if the signal observed was due to identification of a non-specific (i.e. non-SARS-CoV2) genome target (scenario 2). Given our experience with testing environmental samples, the increased likelihood of non-specific targets in environmental compared to human samples, and the very low signal observed (i.e. Ct numbers close to the cut-off of 40), we feel scenario 2 is the most reasonable interpretation of the data.

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.”