June 11, 2020

Big Sky Wastewater Testing Results

Result Summary: Big Sky samples were negative but trending toward positive

Sample Description:

A composite sample of wastewater (1.5 L total) inflow to the Big Sky treatment plant was captured on 6/9/2020 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 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. 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 Sample ID	**Replicate ID**	**Target**	**Ct**	**Potential Genomes per liter**
Inflow_1	Inflow_1.1	N1	NA	NA
Inflow_1	Inflow_1.1	N2	39.6113	573
Inflow_1	Inflow_1.2	N1	37.4776	404
Inflow_1	Inflow_1.2	N2	41.0313	207
Inflow_2	Inflow_2.1	N1	37.3724	439
Inflow_2	Inflow_2.1	N2	39.8631	478
Inflow_2	Inflow_2.2	N1	38.115	242
Inflow_2	Inflow_2.2	N2	40.617	279
Inflow_3	Inflow_3.1	N1	37.9026	287
Inflow_3	Inflow_3.1	N2	40.258	360
Inflow_3	Inflow_3.2	N1	37.7093	335
Inflow_3	Inflow_3.2	N2	41.6319	135

Interpretation:

We detected a concerning signal increase in all but one of the replicate wastewater inflow samples, particularly the N1 target, which unlike previous weeks of testing, was consistently detectable (i.e. above limit of detection). Signal from the N2 target was present but not consistently below the CDC recommended cut-off value of ≤40 Ct numbers. Such results might be expected if the amount of virus in wastewater approached the assay detection limit. Given our experience with testing environmental and wastewater samples and results from previous weeks of testing, we feel these results are consistent with an increasing trend in virus level that should be monitored closely.

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."