

July 2, 2020

## Big Sky Wastewater Testing Results

### Result Summary: Big Sky sample was positive

#### Sample Description:

A composite sample of wastewater (1.5 L total) inflow to the Big Sky treatment plant was captured on 6/30/2020 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 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) 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				Potential Genomes per liter
Sample ID	Replicate ID	Target	Ct	
Inflow_1	Inflow_1.1	N1	36.7313	3928
Inflow_1	Inflow_1.1	N2	38.8569	774
Inflow_1	Inflow_1.2	N1	37.0464	3241
Inflow_1	Inflow_1.2	N2	38.9011	747
Inflow_2	Inflow_2.1	N1	36.1622	5562
Inflow_2	Inflow_2.1	N2	37.9314	1626
Inflow_2	Inflow_2.2	N1	36.0216	6060
Inflow_2	Inflow_2.2	N2	39.5626	439
Inflow_3	Inflow_3.1	N1	FAILED REACTION	NA
Inflow_3	Inflow_3.1	N2	38.9266	732
Inflow_3	Inflow_3.2	N1	36.1818	5495
Inflow_3	Inflow_3.2	N2	39.5035	461

#### Interpretation:

Virus signal for both N1 and N2 targets was observed in all but a single replicate that apparently failed to amplify (this is expected from time to time due to machine and human error). All signals had Ct numbers below 40. These results are entirely consistent with a positive result. The trend in virus levels was decreased from last week's sample, suggesting that the number of cases may be decreasing.

#### 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."