

June 6, 2020

Three Forks Wastewater Testing Results

Result Summary: Three Forks sample was negative

Sample Description:

- 1) A composite sample of wastewater (1.0 L total) inflow to the Three Forks treatment plant was captured on 6/5/2020 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:

Three Forks				Potential Genomes per liter
Sample ID	Replicate ID	Target	Ct	
Inflow_1	Inflow_1.1	N1	NA	NA
Inflow_1	Inflow_1.1	N2	NA	NA
Inflow_1	Inflow_1.2	N1	NA	NA
Inflow_1	Inflow_1.2	N2	NA	NA
Inflow_2	Inflow_2.1	N1	NA	NA
Inflow_2	Inflow_2.1	N2	NA	NA
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:

None of the replicate samples from Three Forks produced a signal in this round of testing. Given our experience with testing environmental samples, there is no evidence of the virus in this sample.