

July 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 7/2/2020 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 should be interpreted with caution, as described below.

The inflow sample was 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:

City name				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	39.18	250
Inflow_2	Inflow_2.1	N2	NA	NA
Inflow_2	Inflow_2.2	N1	39.18	250
Inflow_2	Inflow_2.2	N2	NA	NA
Inflow_3	Inflow_3.1	N1	38.63	376
Inflow_3	Inflow_3.1	N2	NA	NA
Inflow_3	Inflow_3.2	N1	42.89	16
Inflow_3	Inflow_3.2	N2	NA	NA

#### Interpretation:

No signal for the N2 target was detected in any of the sample replicates. In contrast, signal for the N1 target was detected in 4 of 6 samples with Ct numbers in 3 replicates <40. This somewhat consistent signal is concerning, given that no signal for either genome target has been observed in samples from the Three Forks community to date. While there is not enough evidence to call this sample positive, our experience with previous wastewater testing suggests that virus levels may be increasing. Trends in virus levels will be monitored closely to understand whether the signal continues to increase.

#### 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.”