

July 9, 2020

Three Forks Wastewater Testing Results

Result Summary: Three Forks sample was positive

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	37.15	19492.52
Inflow_1	Inflow_1.1	N2	38.14	80096.39
Inflow_1	Inflow_1.2	N1	36.52	29881.03
Inflow_1	Inflow_1.2	N2	43.69	4934.47
Inflow_2	Inflow_2.1	N1	36.23	36344.40
Inflow_2	Inflow_2.1	N2	37.69	100632.17
Inflow_2	Inflow_2.2	N1	36.60	28257.34
Inflow_2	Inflow_2.2	N2	40.29	27148.49
Inflow_3	Inflow_3.1	N1	39.28	4634.80
Inflow_3	Inflow_3.1	N2		
Inflow_3	Inflow_3.2	N1		
Inflow_3	Inflow_3.2	N2		

Interpretation:

Virus signal in the previous week's sample (7/2/2020) was too low to be considered positive. In contrast, signal was detected in all replicates in this week's sample (Note: A problem was encountered when processing Inflow replicate 3, or Inflow_3, such that three of the four technical replicates could not be analyzed). Even though two replicates (Inflow_1.2, N2; and Inflow_2.2, N2) had Ct numbers >40, the presence of virus signal in all replicates is consistent with a positive result. Given no detection in Three Forks until last week (7/2/2020) and increased levels this week, we interpret these data as a positive wastewater sample.

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."