Untreated wastewater can be considered as a community-based excrement sample that represents population’s health status. A person who gets infected will start shedding SARS-CoV-2 RNA from day one from all bodily fluids (saliva, tears, urine, fecal matter, etc.). The person will likely get symptoms on day 8-14 and the positive COVID-19 case would be registered by public health officials even later.

Wastewater surveillance can be used as an additional resource for health officials to monitor and predict SARS-CoV-2 prevalence in the community. The final product of wastewater-based-epidemiology that is of interest to health officials is the lag time i.e., the time difference between SARS-CoV-2 RNA detection in wastewater and the time a person in the community gets tested positive.

The lag time gives the public health officials, hospitals and the government to forecast COVID-19 infections and to prepare in advance.

### Objective and Methods

- **To determine whether wastewater could be used as an epidemiological tool for COVID-19 infections.** If so, what is the lag time period between SARS-CoV-2 RNA detection in wastewater and the time a person in the community gets tested positive for COVID-19.
- **Untreated wastewater samples were collected from the Great Lakes Water Authority (GLWA) Water Resources Recovery Facility (WRRF) located in southeast Michigan between Aug 6, 2020 to Dec 14, 2020.** The WRRF receives wastewater from its service municipalities via three main interceptors: Detroit River Interceptor (DRI), North Interceptor-East Arm (NI-EA), and Oakwood-Northwest-Wayne County Interceptor (ONWI).

Fig 1 shows the areas served by the interceptors. All the three interceptors were weekly sampled in triplicates.

- **Samples were passed through NanoCeram electropositive cartridge filters, eluted with beef extract solution, concentrated, and ultimately RNA was extracted using a QIAGEN VIRAL RNA kit.**
- **A two-step RT-qPCR was used to quantify SARS-CoV-2 RNA from samples using CDC recommended N1 primer and probe. Total of RNA was reverse transcribed using SuperScript RT-qPCR Supermix (Bio-Rad). For each sample, five microliters of cDNA were transferred to a 15 μL reaction mix containing a final concentration of 150 nM of each primer, 125 nM of probe, 1x LightCycler 480 probes master, and sterile nuclease free water.** All reactions were performed in four replicates with the following amplification conditions: denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s and 55°C for 30 s. All qPCR runs included a positive and a negative control.

### Results

- **Fig 2 shows SARS-CoV-2 RNA concentrations in ONWI, NI-EA and DRI Interceptors.**

![Fig 2: SARS-CoV-2 RNA concentrations in ONWI, NI-EA and DRI Interceptors](image)

- **Fig 3 shows observed COVID-19 cases between August 5 to December 15, 2020.**

![Fig 3: Observed COVID-19 cases between August 5 to December 15, 2020](image)

- **Fig 4 shows biweekly confirmed COVID-19 cases in the City of Detroit and Wayne, Macomb and Oakland Counties.**

![Fig 4: Biweekly confirmed COVID-19 cases in the City of Detroit and Wayne, Macomb and Oakland Counties](image)

### Conclusions

- **Early warning of second wave of COVID19 were observed ahead of clinical data reporting.**
- **Statistical analysis validated a lag time of four weeks between observed SARS-CoV-2 RNA concentrations in wastewater and COVID-19 reported cases.**
- **Wastewater could be used as an epidemiological tool to predict the trend of COVID-19 infections.**