



Monitoring Wastewater for SARS-CoV-2 RNA

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Introduction

- Wastewater-base Epidemiology (WBE)
 - Dr. Roberto Cattaneo - Department of Molecular Medicine, Mayo Clinic
- Goal is to monitor the presence SARS-CoV-2 RNA (the causative agent of COVID-19 [1]) in wastewater samples within Rochester, MN.
 - SARS-CoV-2 RNA is shed through fecal matter which can be detected by a PCR-based method [2].
- Monitoring how much RNA is in the wastewater will help predict and prevent future outbreaks

Methods

- Wastewater Reclamation Plant collects six 40 mL samples of clarified wastewater from various locations (Figure 1).
- At Mayo Clinic:
 - Filtration and Concentration:
 - Samples are filtered through 0.7 micron glass filter
 - Then, Concentrated using an Innovaprep Concentrating Pipette
 - Sample goes from 40 mL to ~300 uL (100-fold)
 - Extraction:
 - RNA was extracted using a Qiagen Viral RNA Purification Kit.
 - Detection and Quantitation:
 - Using a digital droplet PCR (ddPCR), the concentration of RNA within a microliter of wastewater is determined (ng/uL)



Figure 1: The six collection sites in Rochester MN

Results

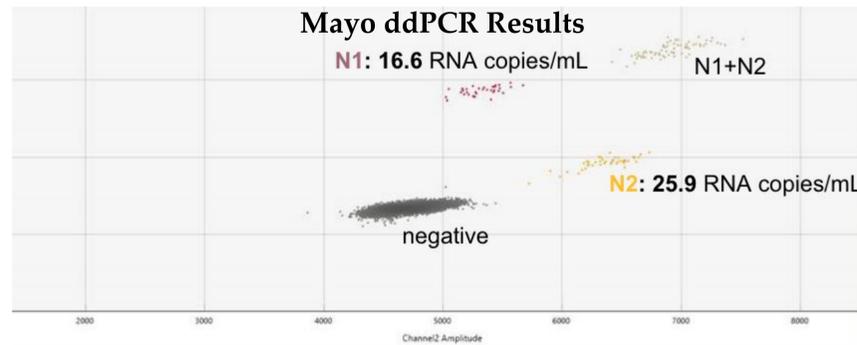


Figure 2: Demonstrates the copies of SARS-CoV-2 RNA per milliliter of wastewater within their respective spectrums.

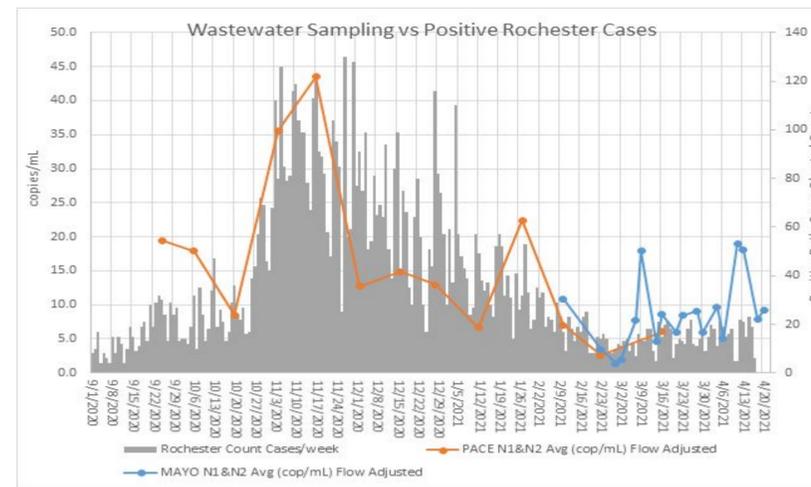


Figure 3: Demonstrates the number of positive cases compared to the concentration of COVID-19 RNA within the wastewater samples.

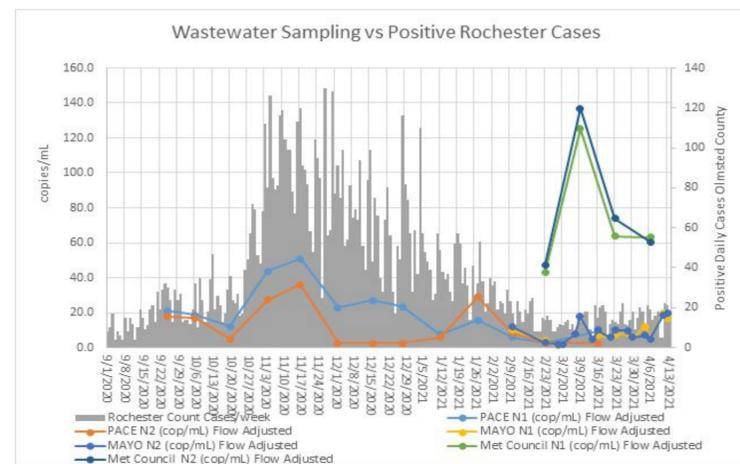


Figure 4: Compares the results of RNA Concentration of Mayo Clinic with the results of the Met Council Lab and the number of positive cases in Rochester.

Discussion/Future Direction

- Figure 2 displays the RNA copies per mL found by our Research at Mayo Clinic which have been comparative to the results of PACE lab.
 - PACE lab uses ddPCR similar to our research.
- Figure 3 demonstrates the weekly concentrations of RNA within wastewater samples from WRP (blue line) compared to the number of positive COVID-19 cases within Rochester (grey bars).
 - The trend of the concentrations similarly follow the trend of the number of positive cases, showing a correlation between the two.
- Figure 4 gives a more detailed comparison to the Met Council lab results which are much higher than the results from Mayo Clinic.
 - The Met Council Labs do not filter their samples where our samples were filtered, which may be the cause of the discrepancy.
 - Due to the filtration, our research may show a loss of copies of RNA.
- Nevertheless, Mayo Clinic and Met Council Lab results have similar trends that correlate with the number of positive cases in Rochester.
- As this project continues, collaboration with the City of Rochester, WRP and Olmsted County Public Health Services will continue to monitor concentration of RNA and to take action to prevent any future outbreaks when the concentrations begin to rise.

References

[1] Aguiar-Oliveira, M. L., et al. (2020). Wastewater-Based Epidemiology (WBE) and Viral Detection in Polluted Surface Water: A Valuable Tool for COVID-19 Surveillance-A Brief Review. *International journal of environmental research and public health*. <https://doi.org/10.3390/ijerph17249251>

[2] Graham, K. E., et al. (2021, Jan. 5). Sars-Cov-2 rna in wastewater settled solids is associated with covid-19 cases in a large urban sewershed. *Environmental Science & Technology*. <https://doi.org/10.1021/acs.est.0c06191>

[3] Beyene, B., Bjork, M., Bjornburg, C., Moyer, A., Turri, W., Sussman, C.