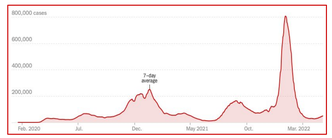
# *Monitoring Infectious Disease with Environmental Surveillance*

*Yash Trivedi, May Thu Aung, Rabia Rasheed, Ryan Ramasray, Dr. Bryan Gibb*

## BACKGROUND

- SARS-CoV-2 is the single stranded RNA virus that is responsible for the worldwide COVID-19 disease pandemic
  - **The virus has caused over 500 million cases and 6.2 million deaths as of April 2022**




New reported cases in the U.S., as per the New York Times. Given the data, the summer looks to be the next wave of cases, given recently inaccurate reporting and waning immunity of vaccinated individuals

- The viral spike protein binds to ACE2 receptors, which are found across the body in different tissue types, but largely infects humans by adhering to receptors in the respiratory tract
- COVID-19 is a disease with implications that extend beyond simple infection, challenging the public health sector and costing the economy trillions in disruptions

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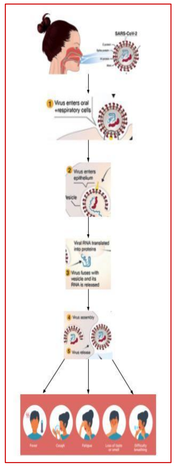
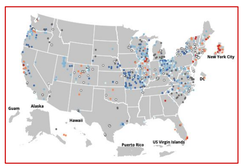
- **Due to the nature of at-home testing and complexity of asymptomatic cases, the ability to accurately quantify and predict infection rates has been diminished**
  - Since the fall of 2021, at-home rapid antigen testing became cheaper and readily available. While these tests work well for individuals at home, the results are rarely reported to public heath agencies
    - This means that official case counts are less accurate now than any time since the start of the pandemic
  - Positivity rates from medical facilities and hospitalizations are leading indicators of cases, but they lag in capturing day to day numbers




Steps of SARS-CoV-2 infection and resulting symptomology

- **One solution is wastewater based epidemiology (WBE)**: which uses biological indicators within sewage as a public health surveillance tool
  - The RNA of SARS-CoV-2 can be detected from fecal matter excreted from infected individuals, hence wastewater treatment facilities are prime locations to collect samples for processing
  - This enables noninvasive monitoring of SARS-CoV-2 in the community. The resolution of these results depends on where and how often samples are collected
    - In efforts to improve university monitoring, we created a research project to establish the methods to conduct wastewater surveillance of SARS-CoV-2 at NYIT
    - Thus, we hypothesized that positive detection and quantification of viral RNA from wastewater samples can be a useful tool for predicting COVID-19 case trends in our area

- The CDC commissioned the **National Wastewater Surveillance System (NWSS)** to build the nation's capacity to track the presence of SARS-CoV-2 in wastewater samples collected across the country
  - NWSS is in many regions of the U.S., with greatest positive sampling in New York City



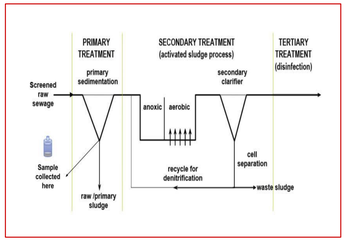

# SARS-CoV-2 Detected in NYIT Wastewater During Omicron Surge

**NYIT onsite wastewater treatment plant (WWTP)**

New York Institute of Technology

## NYIT WWTP

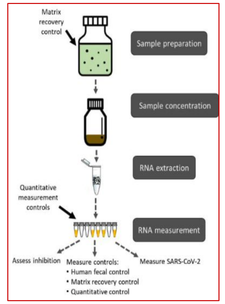
- Every Wednesday during the Spring 2022 semester, **samples from the primary settling tank were collected and brought back to the lab for processing**
  - Primary influent is subject to population level, hence influent levels vary and go to 0 at night
  - Therefore, sampling the settling tank allows us to capture a more accurate, multi-day average for wastewater samples




Schematic of wastewater flow at NYIT wastewater treatment plant (WWTP

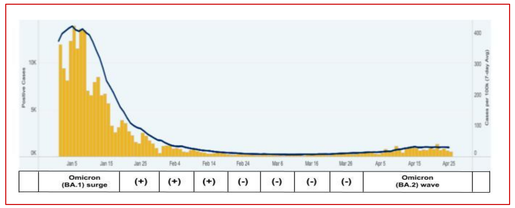
## METHODS

- Sample pasteurization
- Virus concentration with NaCl and PEG
- RNA extraction with TRIzol
- **RT-qPCR**
  - Pepper mild mottle virus (PMMoV) is our positive control due to its abundance in wastewater; therefore it serves as an internal standard for SARS-CoV-2 RNA quantification
  - Primers are for nucleocapsid (N1) gene, which is the standard target for SARS-CoV-2 RT-qPCR due to high sequence conservation across variants




Wastewater processing and RNA quantification methods

## RESULTS




**Our methods detected positive samples in NYIT wastewater in early February, but not late February or March, directly reflecting the Omicron BA.1 surge timeline**

## CONCLUSIONS

- WBE can not only detect, but also correlate the SARS-CoV-2 positive samples to COVID-19 case trends at the population level in real time
  - **These methods can detect cases 2 weeks prior to hospitalization, which means WBE is a powerful tool for predicting looming surges of COVID-19. However, WBE does not extrapolate exact case numbers**
- Environmental surveillance is a useful tool for monitoring infectious disease, and should be taken in context with other diagnostic measures and public health systems to combat the COVID-19 pandemic

## FUTURE DIRECTIONS

- Whole genome sequencing and using spike (S) protein primers will enable targeted variant detection, allowing us to determine which strains and variants are present in larger scale municipalities such as New York City. With further funding and sampling, these methods will allow us to predict future outbreaks and take initiative to combat not only SARS-CoV-2, but other infectious pathogens as well
- **Adapting the qPCR methods, combined with extensive sequencing, we can track evolution of genomic regions of interest among pathogenic agents**. This can be used to springboard our existing study exploring antimicrobial resistance (AMR)