

**High-Confidence, multi-pathogen wastewater surveillance of measles virus, *Mycobacterium tuberculosis*, and *Vibrio cholerae* in Nassau County, New York**

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## Abstract

Wastewater-based epidemiology (WBE) offers a powerful, non-invasive tool for early detection and population-level monitoring of infectious diseases. Building upon the success of SARS-CoV-2 surveillance, we developed and validated a multi-pathogen WBE platform targeting measles virus (MeV), *Mycobacterium tuberculosis complex* (MTBC), *Mycobacterium tuberculosis* (Mtb), and *Vibrio cholerae* in Nassau County, New York. The primary objective was to establish a surveillance system underpinned by a robust Quality Assurance/Quality Control (QA/QC) framework to ensure data reliability for potential public health action, especially during mass gathering events. Our methodology was validated to characterize key performance metrics. RNA and DNA extraction efficiencies and recovery rates were monitored using Bovine Coronavirus and *Enterococcus faecalis* as spiked process controls, respectively. Limits of detection (LOD) were determined to be in the range of 0.3 to 0.7 gene copies/ $\mu\text{L}$  for all targets. The QA/QC protocol incorporated the mandatory use of process controls, extraction blanks, and no-template controls to ensure sample integrity and detect cross-contamination. This platform was deployed during the 2024 ICC Men's T20 World Cup held in Nassau County. During the tournament period, a significant increase in SARS-CoV-2 concentrations was observed in wastewater, a trend that was later mirrored by a rise in clinically reported COVID-19 cases, thus validating the system's responsiveness to real-world transmission dynamics. In contrast, no detections of MeV, MTBC, Mtb, or *Vibrio cholerae* were recorded, indicating these diseases were not circulating at detectable levels in wastewater. This work demonstrates the successful implementation of a high-confidence, multi-pathogen WBE platform capable of functioning as a sentinel system during high-risk events.