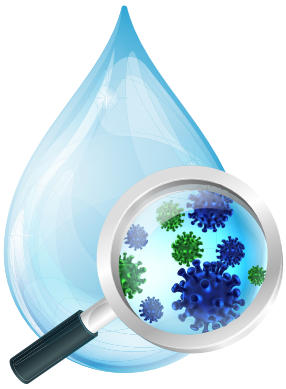
Tech Spotlight
dPCR System

Digital PCR (dPCR) for wastewater surveillance:

Accurate, sensitive monitoring of pathogens



Digital PCR (dPCR)-based wastewater testing is a powerful tool for detecting viral pathogens in a population—even in asymptomatic or presymptomatic individuals. One example is the CDC's National Wastewater Surveillance System (NWSS) for the detection of SARS-CoV2[1]; other respiratory viruses such as influenza A/B and respiratory syncytial virus (RSV) can also be detected and quantified in wastewater using dPCR[2].

One common hurdle to pathogen detection in wastewater is the presence of enzymatic inhibitors in these samples. However, dPCR is highly resistant to many such inhibitors, as the partitioning of each sample into tens of thousands of microreactions effectively reduces their concentration. The **Digital LightCycler® dPCR System** is thus well-suited for wastewater analysis, as this system enables high-volume input and the partitioning of each sample into up to 100,000 reactions.

Monitoring of pathogen-associated DNA and RNA in wastewater provides rapid, localized assessment of infectious disease trends.

Pathogens enter wastewater streams when infected people use the bathroom, wash their hands, or launder their clothing. Sources include residential communities, schools, and hospitals.

Samples are collected prior to treatment at water treatment plants, or collected from intervening points closer to the point of origin.

Collected samples are transported to laboratories

DNA and RNA are extracted and concentrated

Targets are detected and quantified through dPCR. Multiplexing on the **Digital LightCycler® dPCR System** enables the simultaneous detection of up to 6 targets. The **5X concentrated master mixes** increase the volume of sample that can be analyzed per run.

Absolute numbers of target molecules are determined through Poisson calculations.

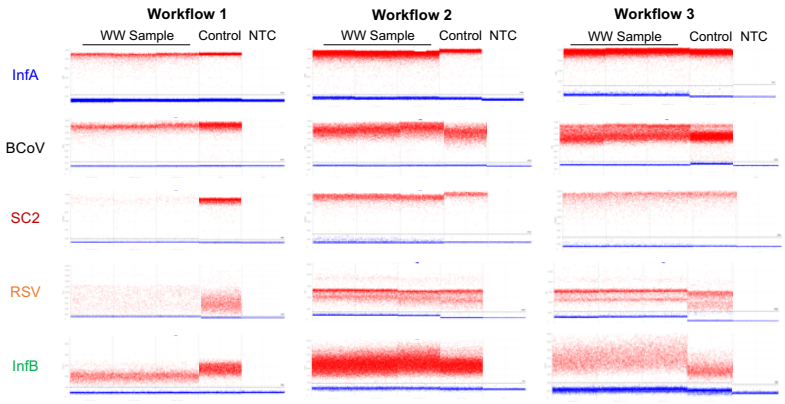
Multiple respiratory pathogens can be co-quantified in a single sample

even in the presence of inhibitors commonly found in wastewater.

Here, raw wastewater was collected from de-identified locations and processed with validated workflows at GT Molecular testing facilities. Viral nucleic acids were then detected using the Wastewater Respiratory Panel from GT Molecular, the Digital LightCycler® dPCR System, and the Digital LightCycler® 5X RNA Master Mix.

As wastewater typically contains many inhibitors that reduce overall PCR efficiency, three workflows were tested to explore the balance between sensitivity and resistance to common inhibitors.

As a positive control, samples were spiked with a known quantity of bovine coronavirus (BCoV). The target pathogens were influenza A (InfA), influenza B (InfB), RSV A/B, and SARS-CoV-2 (SC2).



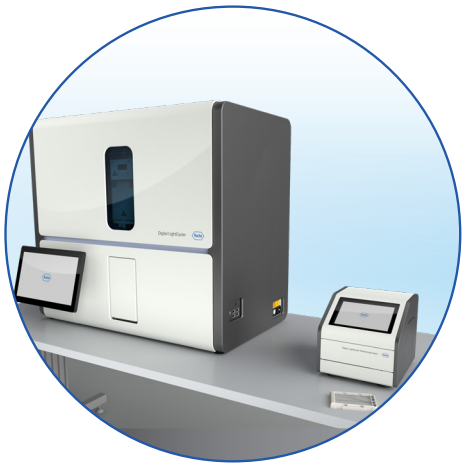
In all three of the wastewater testing workflows optimized by GT Molecular, all of the pathogens were detected in the multiplexed dPCR reactions. This image was adapted from *CF-0181 Digital LightCycler® Upper Respiratory Wastewater Surveillance White Paper R001*[3]. See the White Paper for more detailed explanation of methods and analysis.

The Digital LightCycler dPCR System is a powerful tool for wastewater surveillance, offering:

Robust resistance to inhibitors

Large input volume for testing more sample

High sensitivity



3 Plate configurations

6 Optical channels to enable multiplexing

5X Concentrated master mix

Explore the power, precision, and sensitivity of the Digital LightCycler® dPCR System at **go.roche.com/dpcr**

Visit GT Molecular to learn about their assays at **GTMolecular.com**

1. https://www.cdc.gov/nwss/wastewater-surveillance.html accessed on March 18, 2025
2. https://www.gtmolecular.com/wastewater-and-water-pathogen-pcr-detection-kits/wastewater-respiratory-panel-pcr-kits/ accessed on March 18, 2025
3. CF-0181 Digital LightCycler® Upper Respiratory Wastewater Surveillance White Paper R001. GT Molecular.

The Digital LightCycler® is a Class II US IVD instrument.

MC-US-16815 A726 04/25