Using qPCR Wastewater Testing To Pinpoint Potential COVID-19 Outbreaks

Introduction

Health officials who are aware of how quickly the pandemic landscape can change in terms of local infection rates, virus mutations, and evolving public-health protocols are turning to timely wastewater testing to detect the spread of the SARS-CoV-2 virus as an indicator of COVID-19 spread. This puts wastewater treatment plants (WWTPs) in a unique position to play a key role in supporting better detection, public-health planning, and response to changing threat levels—regardless of vaccination coverage inconsistencies.

Here's how a new twist on a proven technology is making it easier to fulfill that role by delivering quicker, easier, and more reliable onsite detection and quantification capabilities in as little as two hours.

What Is qPCR Testing And What Are Its Benefits?

Polymerase chain reaction (PCR) technology has been used successfully in clinical diagnostic laboratories for several decades, 'amplifying' fragments of nucleic acids—deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)—to aid in the process of identifying and quantifying living organisms. It provides rapid, field-scale detection and identification of wastewater pathogens such as *E. coli*, other fecal indicators, and various nitrifying, phosphorus-accumulating, or filamentous organisms.

A recent adaptation of that technology—quantitative PCR (qPCR) using magnetic beads for easier sample refinement—is now being used to analyze concentrations of SARS-CoV-2 RNA in wastewater samples. Concentrations are measured as genomic copies per milliliter (copies/mL), with the level of absolute concentration expressed in logarithmic proportions—e.g., 4.5x104. Resulting baselines can then be correlated to other observations, such as positive COVID-19 test results and how changing community health guidelines impact the spread of the disease (Figure 1).

Using qPCR within a self-contained, total analytical solution enables WWTPs to control their own in-house testing programs without having to send samples to outside laboratories. This provides a more timely, cost-effective way to generate desired insights and makes it easier for municipalities and institutions—such as universities, nursing homes, penitentiaries, airports, etc.—to exercise better-informed health-policy decisions on protective guidelines and treatment planning.

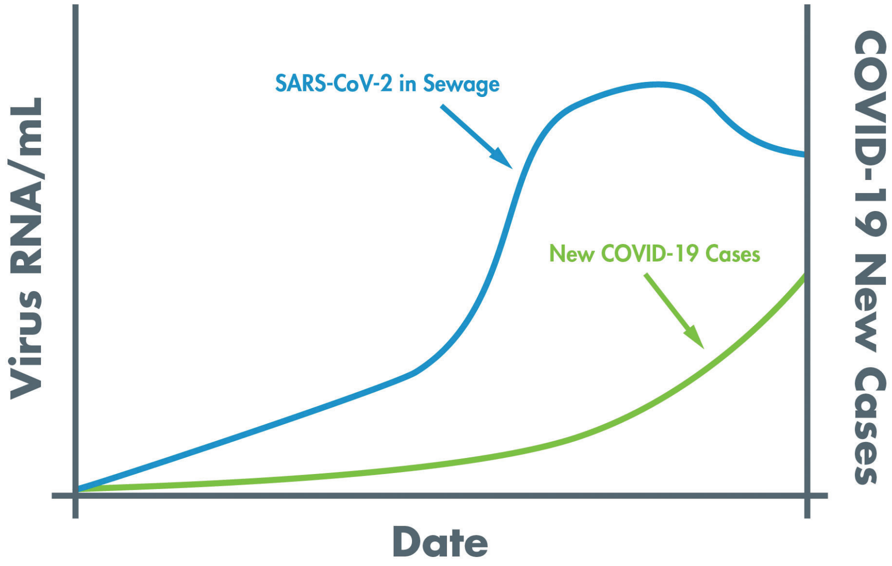


Figure 1. *The lag time in correlation between the presence of SARS-CoV-2 virus in wastewater (blue) and subsequent positive COVID-19 test results (green) among local residents can give public health officials advance notice of increasing risk. qPCR testing that speeds the identification and quantification of such trends in just a few hours enables more timely initiation of new public-health guidelines. (Graphic courtesy of LuminUltra)*

Why In-House Testing?

Testing that compresses turnaround time from sample collection through final result to as little as a few hours provides decision-makers with the greatest lead-time for formulating a strategic response. In-house qPCR testing can also deliver those timely results at a fraction of the cost* for other options—e.g., in about two hours for about $40/test vs. up to seven days at $400 to $600/test for third-party lab testing. Although testing typically takes place at the WWTP facility, wastewater samples can be gathered from anywhere in the collection system. This makes it practical to monitor changes in virus presence and concentration on a system-wide basis or within select subsets of the population—e.g., specific dormitories at a university—and adjust health protocols to the needs of specific populations.

**After instrument purchase.*

Not All In-House Testing Is The Same

The new patent-pending execution of qPCR technology using magnetic beads differs from other approaches by generating faster results at a lower cost per test*, using best-in-class technology to measure even trace amounts of SARS-CoV-2 RNA molecules (Figure 2).

**After instrument purchase.*

	LuminUltra Method	Others
Training Requirements	Low	High – Specialization Required
Equipment Requirements	qPCR Instrument	qPCR Instrument, Centrifuge
Components Required	Complete Kit – from Concentration to Results	Source individual components
Intended Users	Anyone – from in field to lab users	Central High Complexity Lab
Virus Recovery	~20+%	<1-10%
Sample Volume	1 mL	5-100 mL
Limit of Detection	Similar	

Figure 2. *This chart highlights differences between new in-house qPCR testing technology using coated magnetic beads to extract SARS-CoV-2 virus RNA from wastewater samples and other PCR implementations. Benefits include reduced equipment demand and expense, lower training requirements, smaller sample-volume requirements, higher virus recovery rates, and the built-in convenience of a complete kit solution. (Chart courtesy of LuminUltra)*

- **An 'Attractive' Alternative.** The use of silica-coated iron beads that make it easier to attract and separate SARS-CoV-2 RNA from a complex sample matrix is a key differentiator in the cost, time, and simplicity of wastewater testing (Figure 3). Even though this method requires only a 1-mL sample, it can recover 20 to 30 percent of the available viral RNA in that sample—a favorable comparison to other methods that recover only 1 percent or 2 percent.

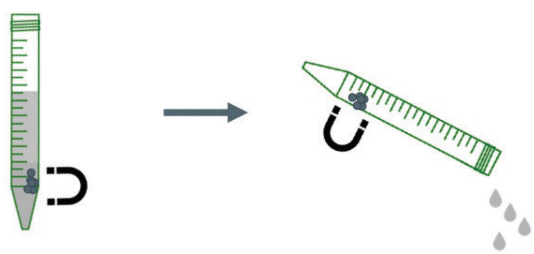


Figure 3. *Using a special coating to bind SARS-CoV-2 particles to small iron beads, than using a magnet to separate those beads from the supernatant, makes the process of recovering and quantifying the presence of SARS-CoV-2 virus from relatively small sample volumes easier and more affordable* to achieve. (Graphic courtesy of LuminUltra)*

**After instrument purchase.*

- **Robustness And Reliability.** Extraneous materials—e.g., drugs, mucus, and other interfering effects—in any sample matrix impact sampling accuracy. The silica-coated beads make it easier to cope with complex wastewater samples, including variables based on geographic conditions—e.g., road salt, industrial wastewater discharges, saltwater inflow and infiltration in low-lying coastal areas, etc.
- **A Quicker Frame Of Reference.** This same qPCR technology's quicker testing, affordability* for more frequent testing, and ability to run smaller samples without compromising results all make it easier to establish initial baselines and track fluctuations against observed impacts in the general population. Field experience from the University of Denver documents how it has had positive effects for adjusting to ever-changing conditions.

**After instrument purchase.*

How It Works

A total-solution kit with a 16-sample qPCR portable unit (Figure 4) makes it easy for individuals familiar with testing for nutrients or organics in wastewater to obtain reliable SARS-CoV-2 results in a fraction of the time required by other PCR methods. Best of all, the new method amplifies small-volume samples for quicker, easier, onsite detection and quantification.

Figure 4. *Using this qPCR bench-top instrument/supplies kit as a total solution to complete in-house SARS-CoV-2 wastewater surveillance in less than two hours eliminates supply chain issues and reduces the cost of testing by 75 percent* vs. other testing methods.*

**After instrument purchase.*

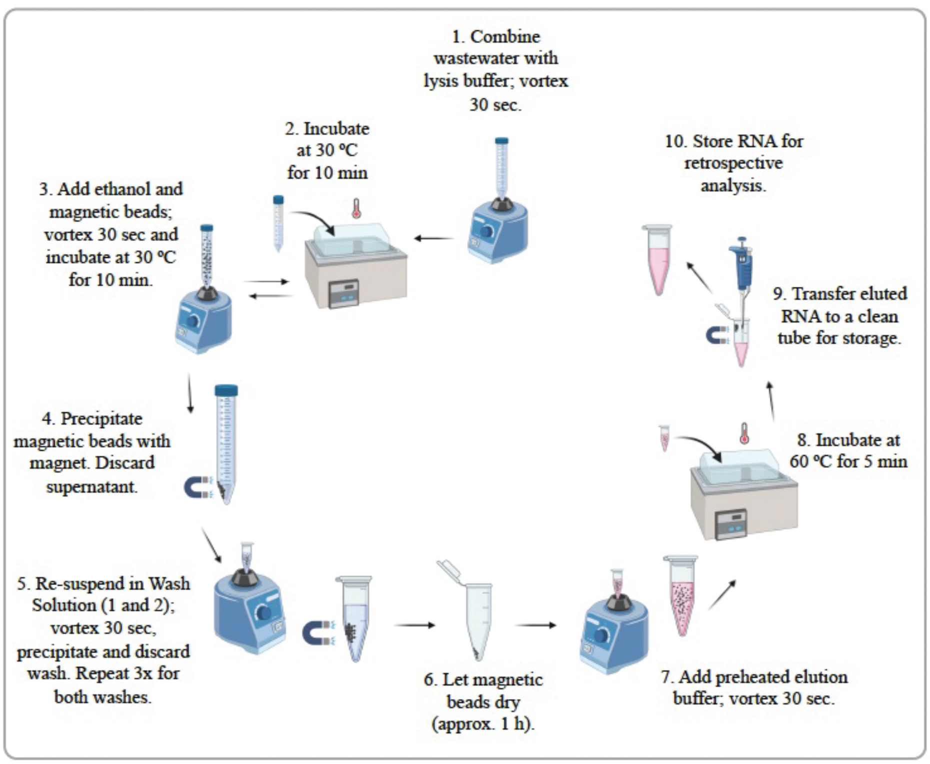


Figure 5. *This complete SARS-CoV-2 detection and quantification process involves a series of simple reagent application steps that can be easily understood by anyone familiar with water testing for nutrients and organics. Whether samples are collected at the WWTP or from targeted sampling sites in the collection system, current WWTP lab personnel can complete the entire process in about two hours. (Source: medRxiv.org)*

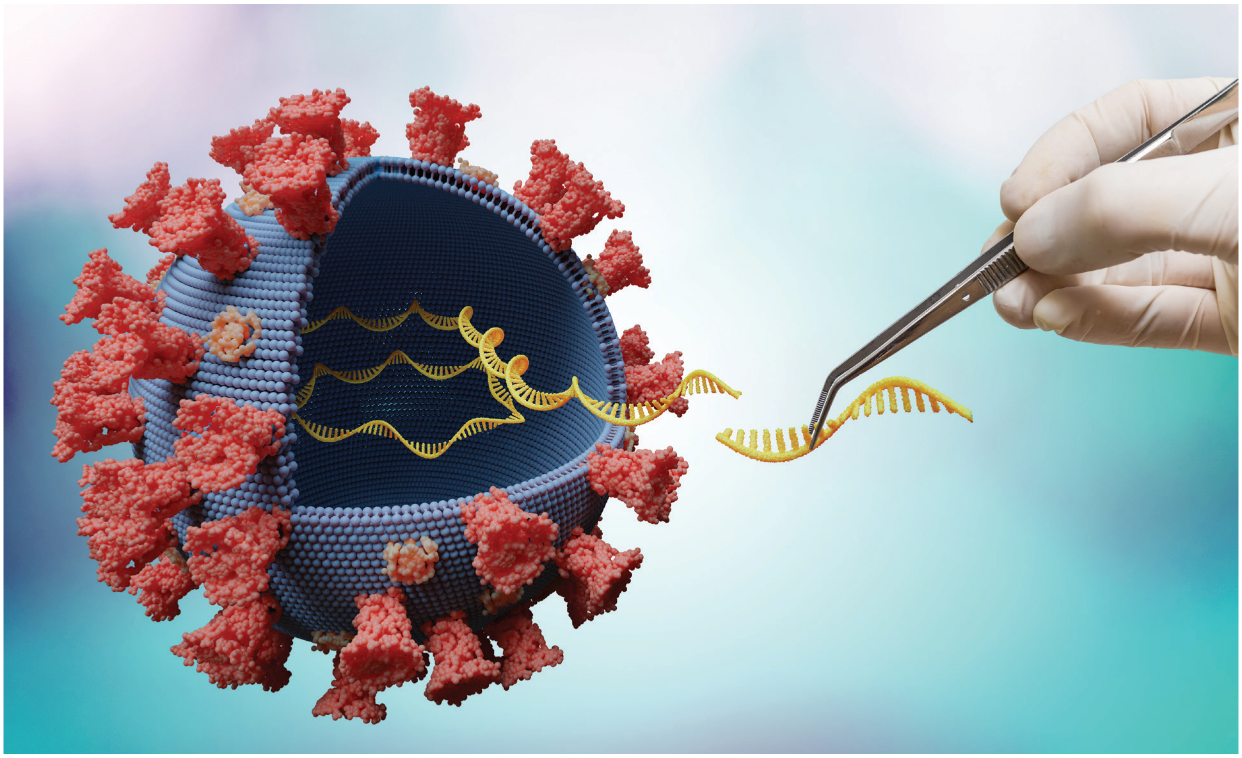
The key to the robust and reliable SARS-CoV-2 sampling with this new qPCR technique is its ability to refine, remove, and measure representative RNA from wastewater samples with a sequential application of four reagents (Figure 5).

- **Use a lysis buffer** to rupture the virus cells and release their RNA into the wastewater solution in a glass vial.
- **Add small, magnetic, iron beads featuring a silica coating** designed to attract and hold RNA from the solution. Once the RNA has bonded to the surface of those beads, the beads can be held to the side of the vial with a magnet while the surrounding liquid is poured off.
- **Cycle through a series of washings** to clear away any remaining impurities before putting an elution buffer into the vial to release the retained RNA from the beads in preparation for qPCR evaluation.

The sequence simplifies and improves the robustness and reliability of the process, concentrating all of the pure RNA into a fraction of the original solution volume—going from 1 mL down 50 µL. It also eliminates the need for special, highly trained personnel or specialized equipment—i.e., expensive centrifuges or vacuum filter equipment. And because it requires minimal training to achieve results in one quarter of the time, it reduces the cost and expands the potential for use.

Taking The Next Steps

Utility and government officials interested in improving response to the COVID-19 pandemic can learn more by requesting information about the new qPCR technology, connecting to The Water Research Foundation's guidance and references, exploring the COVID-19 Water-Based Epidemiology (WBE) Collaborative's map, and visiting the Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) website.



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HACH World Headquarters: Loveland, Colorado USA

United States:	800-227-4224 tel	970-669-2932 fax	orders@hach.com
Outside United States:	970-669-3050 tel	970-461-3939 fax	int@hach.com

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