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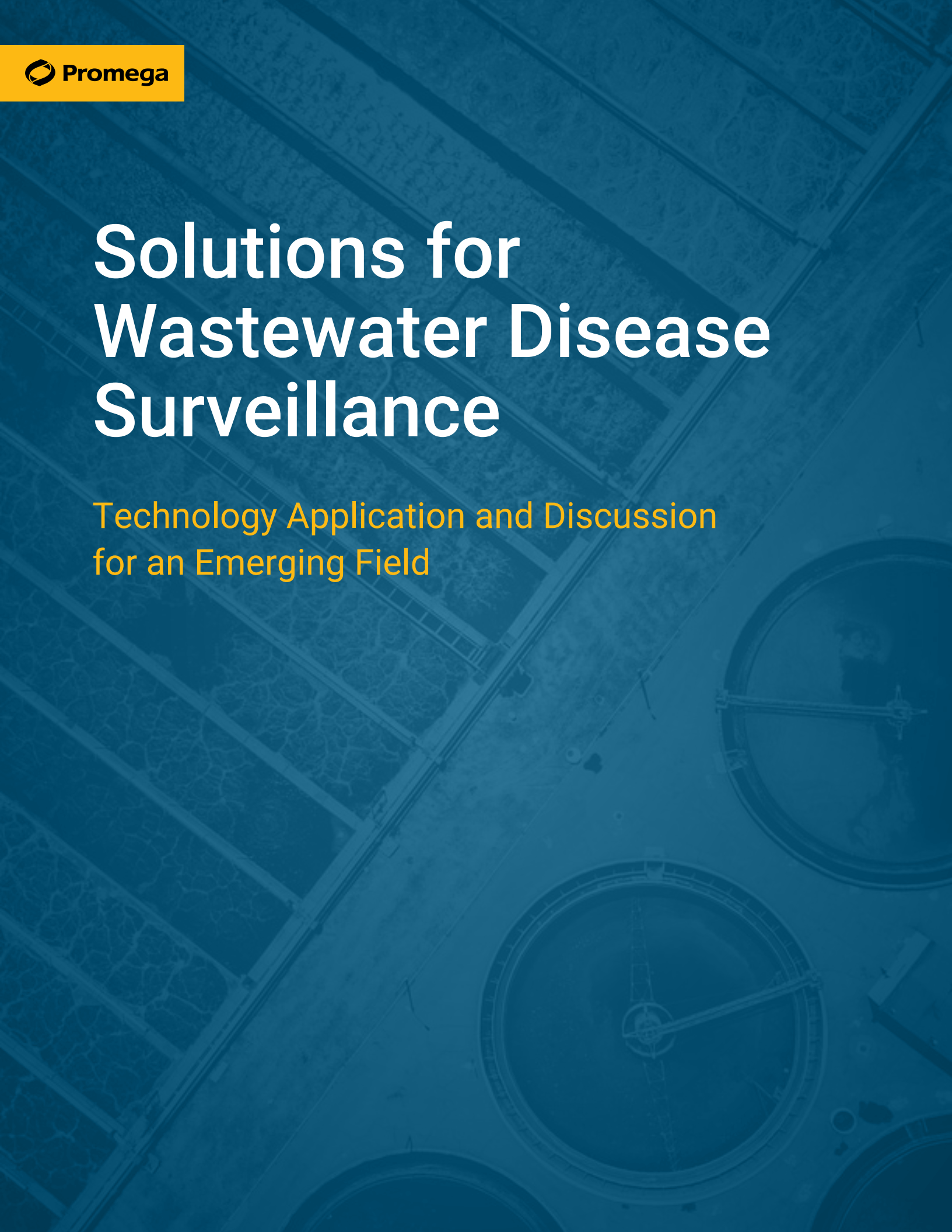
Solutions for Wastewater Disease Surveillance

Technology Application and Discussion for an Emerging Field



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Wastewater Testing Solutions

Catered to Meet Your Needs

30+ years PCR EXPERIENCE

AUTOMATED FORMAT OPTIONS

SAMPLE PREPARATION EXPERT

Your Vision. Realized.

Finding the right partner for your wastewater testing needs can be difficult. Whether you are looking to run wastewater testing in-house or finding a service to run the tests for you, Promega has you covered.

As a company built on delivering high-quality products on time and to your specifications, we have a dedicated team of scientists willing and ready to provide you with expertise, ongoing technical support and quality standards to ensure your institution's success with your products.

Have questions about wastewater testing tools and resources?

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Solutions for Wastewater Disease Surveillance

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01

Introduction to Wastewater-Based Epidemiology

1.1

What is Wastewater-Based Epidemiology

Wastewater-based epidemiology (WBE) is the analysis of wastewater to monitor public health. The term first emerged in 2001, when a study proposed the idea of analyzing wastewater in sewage treatment facilities to determine the collective usage of illegal drugs within a community. At the time, this idea to bridge environmental and social sciences seemed radical, but there were clear advantages. Monitoring wastewater is a nonintrusive and relatively inexpensive way to obtain real-time data that accurately reflects community-wide drug usage while ensuring the anonymity of individuals.

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1.2

History of Implementation

WBE, or sewershed surveillance, is an approach using analysis of wastewater to identify the presence of biologicals or chemicals relevant for public health monitoring. WBE is not new, as wastewater has previously been used to detect the presence of pharmaceutical or industrial waste, drug entities (including opioid abuse), viruses and potential emergence of super bugs. In fact, several countries have been successful in using this method to contain polio and hepatitis A outbreaks.

This idea was realized in a 2005 study in which scientists collected sewage water in major Italian cities to estimate local cocaine usage. Traditionally, cocaine usage was estimated using population surveys, consumer interviews, medical records and crime statistics, all of which can be largely biased. The data they obtained from wastewater surveillance was eye-opening, revealing a much higher level of average daily cocaine usage than previously determined. Now, wastewater is used to estimate local drug use in many countries, including cities and universities in the US.

In addition to monitoring drug use, WBE has been used for decades to monitor viral diseases. Viral genetic material can be detected in human feces for days or even weeks before the onset of patient symptoms. This makes wastewater monitoring the perfect early warning system for preventing outbreaks.

In Israel, a wastewater surveillance program to monitor polio outbreaks has been in place since 1988, which involves monthly sampling of 8–10 sewage treatment facilities in populated areas. In 2013, the polio virus suddenly reappeared in sewage water, alerting health officials to initiate a nationwide vaccination campaign. Because of the early warning, a potentially disastrous outbreak was prevented. Other studies demonstrated how wastewater can be used to detect various strains of norovirus and hepatitis viruses.

Tracking COVID-19

Fast-forward to 2020, the COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has further propelled WBE into the spotlight. Many believe that sewage surveillance is the most practical method for long-term monitoring of SARS-CoV-2 outbreaks along with other potential diseases and pathogens. This approach is more cost-effective compared to large-scale clinical diagnostic testing and can detect viruses like influenza even before symptoms occur, meaning public health officials can be proactive rather than reactive when controlling outbreaks.

In March 2020, researchers at the KWR Water Research Institute found the presence of SARS-CoV-2 RNA in wastewater samples collected near the Schiphol airport in Amsterdam and several other sites in the Netherlands. The result came within a week after the first case of COVID-19 in the country was confirmed. This study opened the door to the possibility of using wastewater-based epidemiology to determine population-wide infections of COVID-19.

The idea of using wastewater surveillance to monitor COVID-19 long-term has spread globally. In March 2021, the European Commission recommended that all member countries put in place a national wastewater surveillance system as soon as possible. This marked an important strategy to monitor the spread of SARS-CoV-2 variants and other emerging pathogens in the future. Similar efforts by the US Centers for Disease Control in conjunction with state health labs have resulted in a broad effort to monitor the RNA levels of wild-type SARS-CoV-2 and variants of concern across the country.

In 2022, a dashboard by the University of California Merced showed the extent of wastewater monitoring globally. Over 280 universities in 68 countries and over 3,500 sites are monitoring their wastewater for SARS-CoV-2. The number of participating institutions continues to increase for public health monitoring efforts.

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1.3

WBE as an Early Warning System

Although WBE cannot identify which individuals have been infected, it has several advantages compared to patient testing. For example, it is much more cost-efficient in obtaining population-wide data. Another advantage is early detection. A study from China showed that SARS-CoV-2 RNA can be detected in human feces a few days to a week ahead of the onset of symptoms. This viral RNA eventually ends up in the sewage system. Another study by Yale University demonstrated that monitoring SARS-CoV-2 viral RNA concentration in sewage water can

Wastewater Video

Click to view how WBE can be an affordable disease surveillance system in a community and serve as a beneficial approach for cities and institutions.

Watch Video

predict COVID-19 outbreaks 7 days before individual patient testing and 3 days before hospital admissions. The researchers concluded that WBE is a leading indicator to identify hotspots in a localized region or population. As a result, WBE can serve as a low-cost, early warning system to identify new outbreaks, trends in current outbreaks and prevalence of infections.

Wastewater surveillance data can complement clinical testing and contact-tracing efforts within a population. This data informs whether public health officials need to take appropriate actions on containment or relax restrictions. As a result, local officials can act promptly, balancing the risks of a public health crisis versus business as usual, which is essential for local economic viability.

1.4

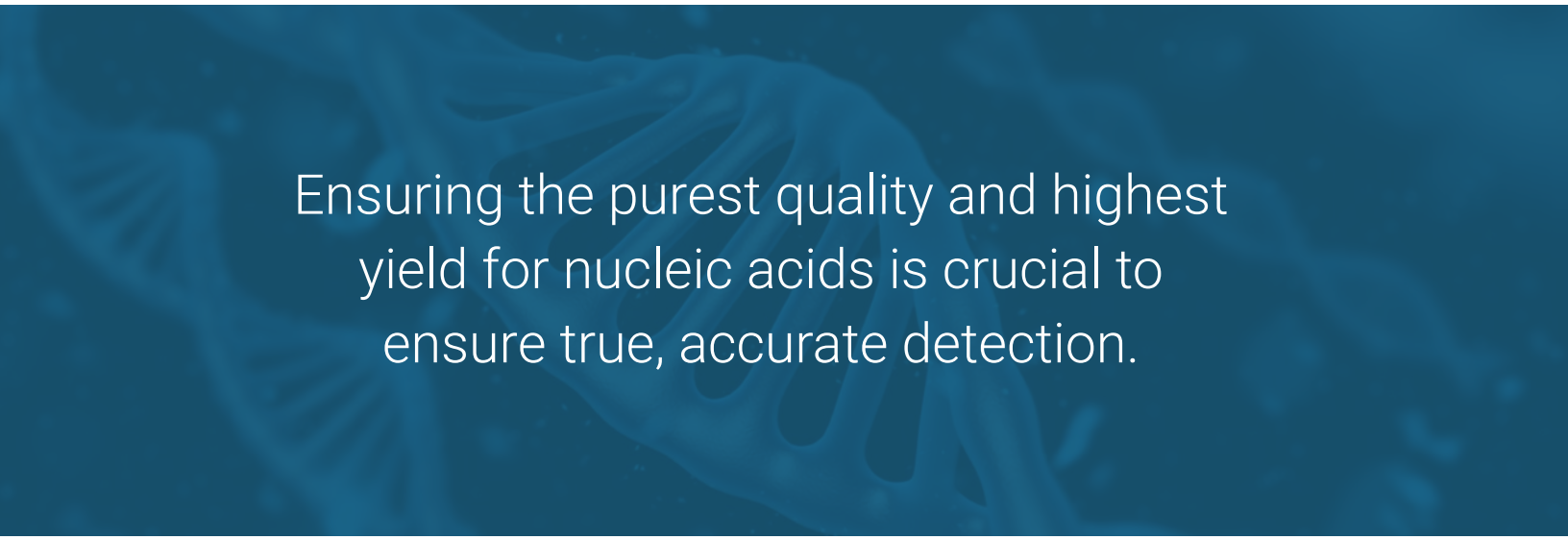
Challenges in Monitoring

Wastewater is a notoriously difficult sample matrix to work with for molecular detection. The challenges range from identifying the best sampling locations to difficulties associated with sample preparation. Wastewater represents a mixed community of different organisms, some in high abundance and others in low abundance. As a result, any bias in downstream applications may reduce confidence in targeted biomarkers for specific organisms.

Difficulties with Sample Preparation

With sample preparation representing a huge portion of the workflow, this area is most affected by compounds within wastewater, including polyphenols and heavy metals. These compounds act as inhibitors that bind to the backbone of nucleic acids, which can prevent sequencing and PCR-based applications. Certain detergents such as Quaternary Ammonium Compounds (QACs) in wastewater matrices and environmental DNases and RNases can degrade nucleic acids and make identifying low-abundance targets even more burdensome.

Even beyond inhibitors, the initial sample matrix is complicated enough to concentrate down for nucleic acid purification. Not losing nucleic acid signature is a challenge, and with certain viral and bacterial targets in low abundance initially, the resulting solution necessitates high volumes of wastewater to process. As a result, methods that are not able to concentrate effectively and efficiently are an impediment to any workflow.



Low Target Abundance

Another commonly overlooked challenge in wastewater analysis for monitoring biologic material and even organic metabolites is how often they occur naturally in the environment. Ultimately, during times when the prevalence of a pathogen is low in a circulating population, specific assays will fall under a limit of detection (LoD), or the lowest concentration of analyte that can be measured in a test. As a result, ensuring the purest quality and highest yield for nucleic acids is crucial to ensure true, accurate detection.

References

1. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, Yang Y, Liu B, Wang W, Wei C, Yang J, Ye G, Cheng Z. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol. 2020 Jul;92(7):833-840. doi: 10.1002/jmv.25825. Epub 2020 Apr 25. PMID: 32243607.

2. Jordan Peccia, Alessandro Zulli, Doug E. Brackney, Nathan D. Grubaugh, Edward H. Kaplan, Arnau Casanovas-Massana, Albert I. Ko, Amyn A. Malik, Dennis Wang, Mike Wang, Daniel M. Weinberger, Saad B. Omer SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics, medRxiv 2020.05.19.20105999; doi: https://doi.org/10.1101/2020.05.19.20105999

3. FQ Wu, A Xiao, JB Zhang, XQ Gu, WL Lee, K Kauffman, WP Hanage, M Matus, N Ghaeli, N Endo, C Duvallet, K Moniz, TB Erickson, PR Chai, J Thompson, EJ Alm, SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases, medRxiv 2020.04.05.20051540;doi: https://doi.org/10.1101/2020.04.05.20051540

4. Subhanjan Mondal, Nathan Feirer, Michael Brockman, Melanie A. Preston, Sarah J. Teter, Dongping Ma, Said A. Goueli, Sameer Moorji, Brigitta Saul, James J. Cali, Promega Corporation, 5430 E Cheryl Pkwy, Fitchburg, WI 53711, United States of America, A direct capture method for purification and detection of viral nucleic acid enables epidemiological surveillance of SARS-CoV-2, Science of the Total Environment 795 (2021) 148834

5. Robotto, A. et al. (2021) Wastewater-based SARS-CoV-2 environmental monitoring for Piedmont, Italy. Environ Res. 203, 111901.

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02

Streamlining a Solution

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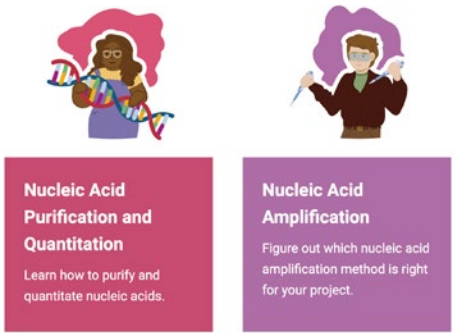
2.1

A Turn-Key Solution for Wastewater Analysis

To address the challenges and support the global demand for wastewater testing, Promega scientists have developed a family of kits that offer labs an end-to-end solution for viral detection in wastewater. Our workflow has been implemented in surveillance projects globally and is proven to deliver high-quality, pure nucleic acids at a greater yield, to ensure that sensitive and low-abundance targets are detected.



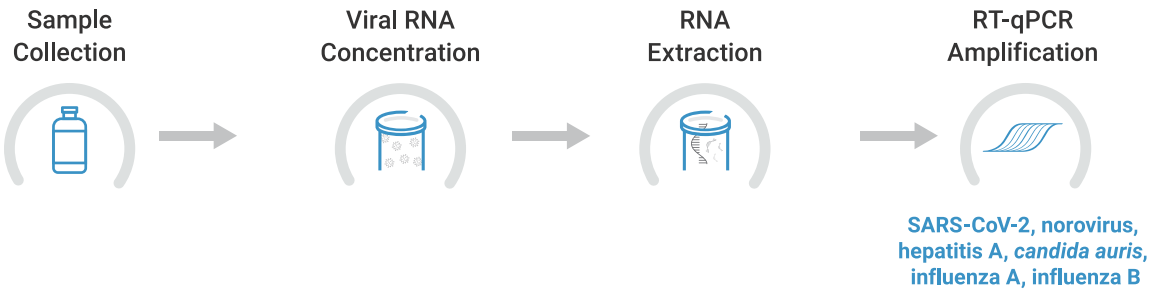
Resource Center - Lab Essentials

Whether you need a quick technique refresher or are looking for support, our Resource Center offers a collection of resources like webinars, blogs and videos related to common molecular biology techniques that would be helpful for pursuing WBE applications.

Learn More

Nucleic acid purification is the center point of wastewater analysis and is one of the most common spots where challenges arise. Whether it be DNA or RNA, the process of purifying or extracting nucleic acids starts with the same five steps, no matter the sample type.

Samples all progress through a common set of manipulations during purification: lysis, clearing, binding, washing and elution. Optimization of extraction methodologies is key for success, especially with challenging sample types and demanding downstream applications. Post-purification, wastewater samples can be amplified via the Polymerase Chain Reaction (PCR). Whether quantitation of a purified gene of interest is DNA-based or RNA-based will determine if qPCR or RT-qPCR is necessary. For review on the important steps in nucleic acid amplification and the considerations for successful amplification, visit our resource center.



The workflow for wastewater disease surveillance starts from sample collection at the site. From there, the process includes a concentration step using a preferred methodology. For ease and simplicity, the Promega total nucleic acid (TNA) purification kits combine both direct extraction (using either silica column-based concentration or magnetic bead-based concentration) and purification. The purified RNA can be paired with a detection system such as RT-qPCR to amplify gene targets utilizing respective primers and probes.

The PCR-based method commonly used for detecting viral RNA in wastewater is very similar to what is used in clinical or research labs. However, the complexity of wastewater samples brings some additional challenges. Sample preparation and ultimately how viral RNA is purified is crucial for successful detection. Promega offers two kits for viral nucleic acid concentration and extraction from wastewater: the Wizard® Enviro Total Nucleic Acid Kit and Maxwell® RSC Enviro Total Nucleic Acid Kit.

Both use a unique filter-based direct capture method that can concentrate total nucleic acid faster than existing precipitation methods. The Wizard® Kit includes minicolumns for manual purification; the Maxwell® RSC Kit allows automated total nucleic acid purification designed to work with Maxwell® Instruments, which can automatically process up to 48 samples at once. For labs that require higher throughput, products utilizing bead-based concentration compatible for plate-based formats supported by liquid handlers are also available (please contact applied@promega.com for more information).

Multi-Panel Detection of Pathogenic Organisms

With these new extraction kits, you can go from sample to purified nucleic acid in as little as two hours. The purified nucleic acid can be used directly for amplification using one of several Promega downstream detection kits available for RT-qPCR analysis

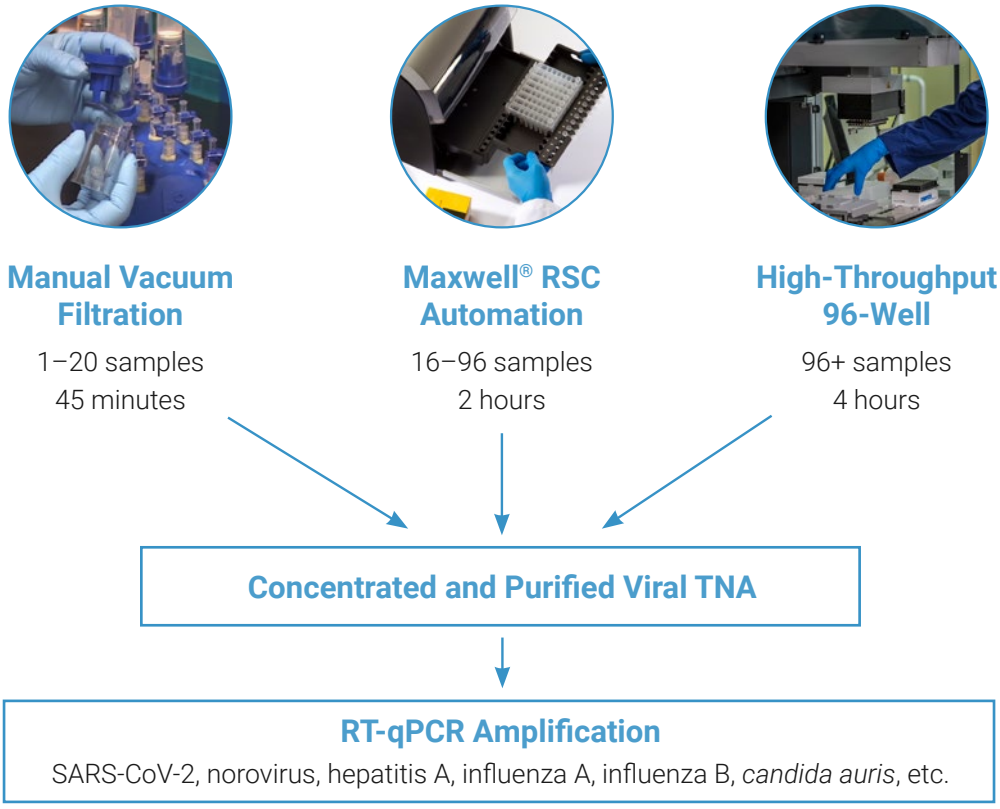
Total Nucleic Acid Protocol Videos

Want to learn more about the direct capture method for processing wastewater samples? Click here to see either Promega's option for manual purification or automated purification methods on the Maxwell. Whether your group is processing one sample or 100 samples, we have a solution.

Learn More

specific to SARS-CoV-2 detection in wastewater. These kits include primers and probes to detect SARS-CoV-2 (N1, N2 or E targets) as well as inhibition controls, quantification standards and a pepper mild mottle virus (PMMoV) internal process control. The RT-qPCR master mix allows sensitive detection and quantification even in the presence of reverse transcriptase inhibitors or PCR inhibitors common in wastewater samples. Primer and probe sets for SARS-CoV-2 variants of concern are also available.

Likewise, as the demand to monitor additional pathogenic organisms has grown, additional detection kits developed by Promega feature norovirus and hepatitis A as individual targets. As methods for detecting viral RNA in wastewater continue to advance, additional WBE programs will be implemented globally to help control and prevent outbreaks of COVID-19 as well as future viral diseases.



Depending on wastewater testing goals, a customized solution from manual-to high-throughput is achievable. Although chemistry changes are involved, fundamental principles involving concentration, purification and detection are required across all wastewater testing workflows.

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2.2

Wastewater Insight from an Expert

A Senior Scientist's Take on Sample to Solution for Wastewater

In this video, senior scientist Subhanjan Mondal explains the importance of having a sample-to-solution answer for wastewater monitoring of SARS-CoV-2. Wastewater presents a wealth of information that gives a snapshot of an entire community. No matter how granular or broad the testing, a streamlined and simple workflow is the key to ensuring consistent and unbiased data.

Play Video

2.3

Service for Monitoring Needs

Wastewater Testing Services in the United States

Depending on your needs for wastewater testing, you may consider several factors on whether it is more practical to process wastewater in-house or use a service. Research-trained personnel, reagents and space for equipment like thermal cyclers are all components necessary to begin testing. If any of these components becomes a limiting factor, you might prefer to consult a trusted partner for routine screening.

Promega offers wastewater analysis testing as a service in the United States to help communities as well as institutions monitor their wastewater. As this field expands and methods are widely adopted, the vast amount of information that can be ascertained from wastewater analysis will help communities and institutions alike address public health issues.

Pathogen Detection Report

Collection

Collection kits designed for routine sampling and ease. Simplify, collect and send in marked packaging.

Analysis

Scientific experts will take care of the rest, utilizing a nucleic acid purification approach and RT-qPCR-based format to determine concentrations of microbial targets of interest.

Reporting

Results delivered in <48 hours in a format that fits your group's needs.

Monitor wastewater for your institution with the ease and versatility of the Promega testing service. From SARS-CoV-2 to influenza, norovirus or hepatitis A, we can help deliver high-quality, real-time data for microbial concentrations.

*Service only available in the United States

03

Specific Use Cases for Surveillance

3.1

Academic Success and Tips for Starting a Workflow

Whether the data is used internally or with hopes of publishing, there are several important considerations for choosing a wastewater screening workflow, whether for academic or research purposes. These include the need for accuracy, reproducibility, efficiency and affordability.

One example of an academic institution improving these core areas for wastewater analysis is the Vienna University of Technology. Initially, the group utilized a manual concentration and purification approach for total nucleic acid. These initial protocols included clunky, time-intensive precipitation methods. While this methodology is feasible for fewer than five samples, once sample volumes started increasing to over 40 samples a day, they found that manual extraction from start to finish took three days.

The group saw their sample volumes as a chance to pivot to automation and increase efficiency and reproducibility, ensuring more consistent results. The Maxwell® RSC Instrument offered the advantage of decreasing human error while increasing throughput. As a result, Vienna University of Technology shortened their entire workflow to a single day.

Quick Automated Purification
Learn the automated and streamlined process Promega created utilizing the Maxwell® RSC Enviro TNA Kit to purify wastewater samples in under 90 minutes.

Learn More

Helpful Tips

Here are some helpful tips to reduce costs, streamline workflows and increase the quality of data. Have a tip you used in your workflow? Let us know at: applied@promega.com

Tip #1 – Automate When Possible to Save Time

With liquid handlers growing more popular for nucleic acid purification, even wastewater samples can be automated to reduce hands-on time. The Maxwell® RSC Instrument is a perfect benchtop solution for a semi-automated purification solution. Simply concentrate wastewater samples and load onto the Maxwell with a scripted workflow.

Tip #2 – Reduce Bias with Proper Controls

Incorporate products such as inhibition controls to determine unusual standard deviation between samples. With inhibitors, these challenges can be accounted for by including an Internal Amplification Control (IAC) providing information on reverse transcriptase and DNA polymerase performance in a one-step RT-qPCR amplification.

Tip #3 – Track the Pepper Mild Mottle Virus for Long-Term Screening

While spike-in controls work well for initial methodology evaluation, for long-term routine monitoring they can be tedious and unnecessary. PMMoV is a naturally abundant target in wastewater, and its concentration rarely fluctuates even during dilution events with rainfall. As a result, it's a useful internal process control (IPC) to normalize data.

Tip #4 – Increase Reproducibility with Extraction Replicates

Often overlooked, extraction replicates can be incorporated into workflows to determine the consistency of processing samples from the same location. Replicates have the added benefit of determining the deviation attributed by the methodology performed.

Tip #5 – Save Both Time and Costs by using Promega Purification and PCR Technology

Whether it be manual purification kits, such as the Wizard® Enviro TNA Kit or automated purification on the Maxwell® RSC Instrument using the Maxwell® RSC Enviro TNA Kit, Promega has quick and affordable validated methodologies.

Wastewater Knowledge Fact

The University of Arizona is one prominent group that was able to detect asymptomatic cases of SARS-CoV-2 in dorm wastewater and prevent additional infections thanks to their routine screening program.

3.2

City and Business Adoption

The use case for the adoption of WBE in cities can be seen in studies conducted in areas such as San Diego County during the pandemic. At scale, community monitoring of SARS-CoV-2 in wastewater bypassed the bottlenecks associated with diagnostic screening. With asymptomatic cases less likely to be detected with patient testing, WBE presents a more holistic, unbiased view of trends in an area. Even more importantly, real-time tracking of viral concentration in wastewater allows estimates, which can then be correlated to clinical cases. Often, this wastewater data serves as a leading indicator.

As a result, there is huge value for cities, institutions and businesses to spot a potential surge in cases before people get tested. Through routine monitoring, cities and businesses can ensure that those they serve are alerted to the spread of a communicable disease.

3.3

Words from Wastewater Treatment Plants

Wastewater utilities and operating plants are responsible for treating water before it is sent for reuse or expelled back into natural water reservoirs. Utilities collecting and sending samples to labs or processing samples internally need access to a simple workflow that accounts for limited resources such as personnel and time. Promega offers an intuitive and accessible workflow for those facilities looking to begin testing. Whether it be assistance with molecular biology techniques or expertise, Promega is eager to help.

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3.4

Public Health Implementation

Combination of Clinical and Wastewater Data

Public health labs as well as hospitals and emergency care facilities are often concerned with clinical data. More importantly, wastewater testing serves to augment the narrow scope of information that clinical data provides, because there can be testing delays as well as additional limitations.

The Houston Health Department is one important case of this coupled effort as trends were noticed in both wastewater and clinical data for certain ZIP codes. With both pieces of the picture, the health department was able to act in communities experiencing increased cases, whether through education campaigns or direct intervention. Through wastewater testing, the Houston Health Department was able to target as many as 74,000 individuals to prevent additional infections through the allocation of resources such as personal protective equipment (PPE).

04

What's Next for the Field

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4.1

Multi-Omics Revolution

Beyond pathogen detection, the future for wastewater monitoring will be one of a multifield approach. Multifield monitoring adds to the scope of information that can be collected for a sample to provide insight on a sample's genome, proteome, transcriptome, metabolome and metagenome. Separated, these fields illustrate just a piece of the puzzle for a particular sample type, but together the fields provide a wealth of information. In particular, wastewater can be seen as a digital fingerprint of the urban gut with everyone in that area contributing to the data. Utilizing a multifield approach, the extent of the information collected might range from detecting concerns for antimicrobial resistance, understanding illicit drug usage and gauging emerging diseases.

Wastewater Knowledge Fact

Of all global wastewater treatment facilities, the District of Columbia has the largest. It can treat up to one billion gallons of wastewater a day!

Information collected might range from detecting concerns for antimicrobial resistance, understanding illicit drug usage and gauging emerging diseases.

The ability to monitor metabolites for drug usage and excreted compounds can inform our understanding of how diseases (both communicable and noncommunicable) are circulating in an area. For instance, the presence of medications in wastewater, such as those that treat allergies or heart conditions, may suggest surges in these conditions in a given community. Traditional molecular detection methods for wastewater have been predominantly focused on PCR and sequencing. As research advances, other tools and technologies such as mass spectrometry can be combined to reveal more valuable data.

4.2

What's Next for Wastewater Surveillance

The field of WBE is continuously evolving. The next steps include adding panels to monitor endemic respiratory pathogens such as influenza A and influenza B strains as well as respiratory syncytial virus (RSV). As the world moves beyond the COVID-19 pandemic, the possibilities for monitoring these common strains that emerge year after year is the next hurdle for supporting public health and safety.

Similarly, the field looks to explore the growing concern of super bugs, which are resistant to multiple antimicrobial treatments. Wastewater screening presents the unique opportunity to see background levels of emerging antimicrobial resistance. Ultimately the collective efforts of both private and public institutions are necessary, and getting these priorities in line will benefit local and global communities.

Wastewater surveillance also opens the door for preventive screening in places like hospitals, nursing homes and even educational institutions.

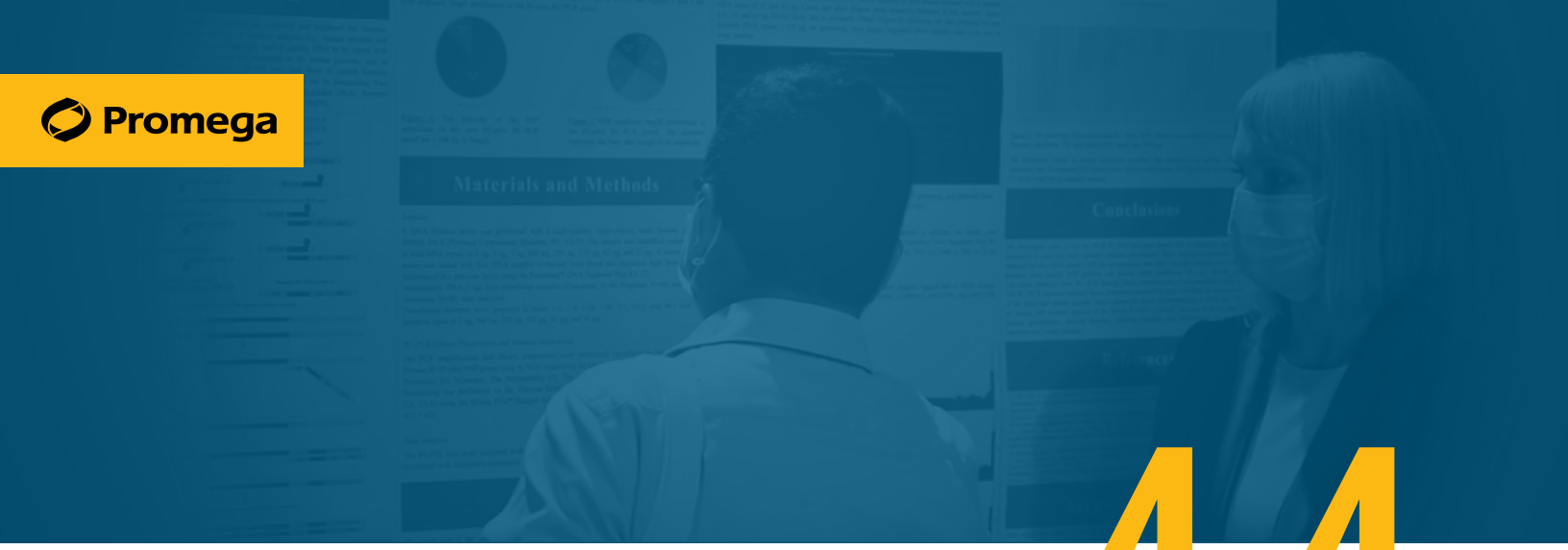
The possibility of implementing preventive strategies based on wastewater surveillance would directly benefit the travel industry. From adoption in aviation to hotels and tourist locations, city officials can get a better understanding of any potential concerns. The first proofs of concept for monitoring in global tourism have already begun. Wastewater surveillance also opens the door for preventive screening in places like hospitals, nursing homes and even educational institutions, typically “hotspots” of activity where WBE could be a useful tool.

4.3

How Can You Get Involved

There are several ways to get involved in wastewater testing. In the United States, the CDC is still actively encouraging participation in the National Wastewater Surveillance System. To date, this program has supported over 560 counties across 44 states including Washington, D.C. The treatment plants in counties involved in this program monitor roughly 20% of the US population. To learn more, visit the CDC's webpage for Waterborne Disease and Outbreak Surveillance Reporting or contact nwss@cdc.gov.

For those who want to start their own wastewater surveillance in their city or area, attending municipal meetings, proposing grant submissions and raising concerns at city ordinance and local events to voice support for wastewater monitoring can bolster this movement. For those in a region outside the United States, please contact applied@promega.com for existing opportunities and programs.



4.4

Community Efforts

Listed below are some of the contributions to the WBE efforts that have been integral in this emerging field. These publications support further study toward improved workflow performance for wastewater monitoring.

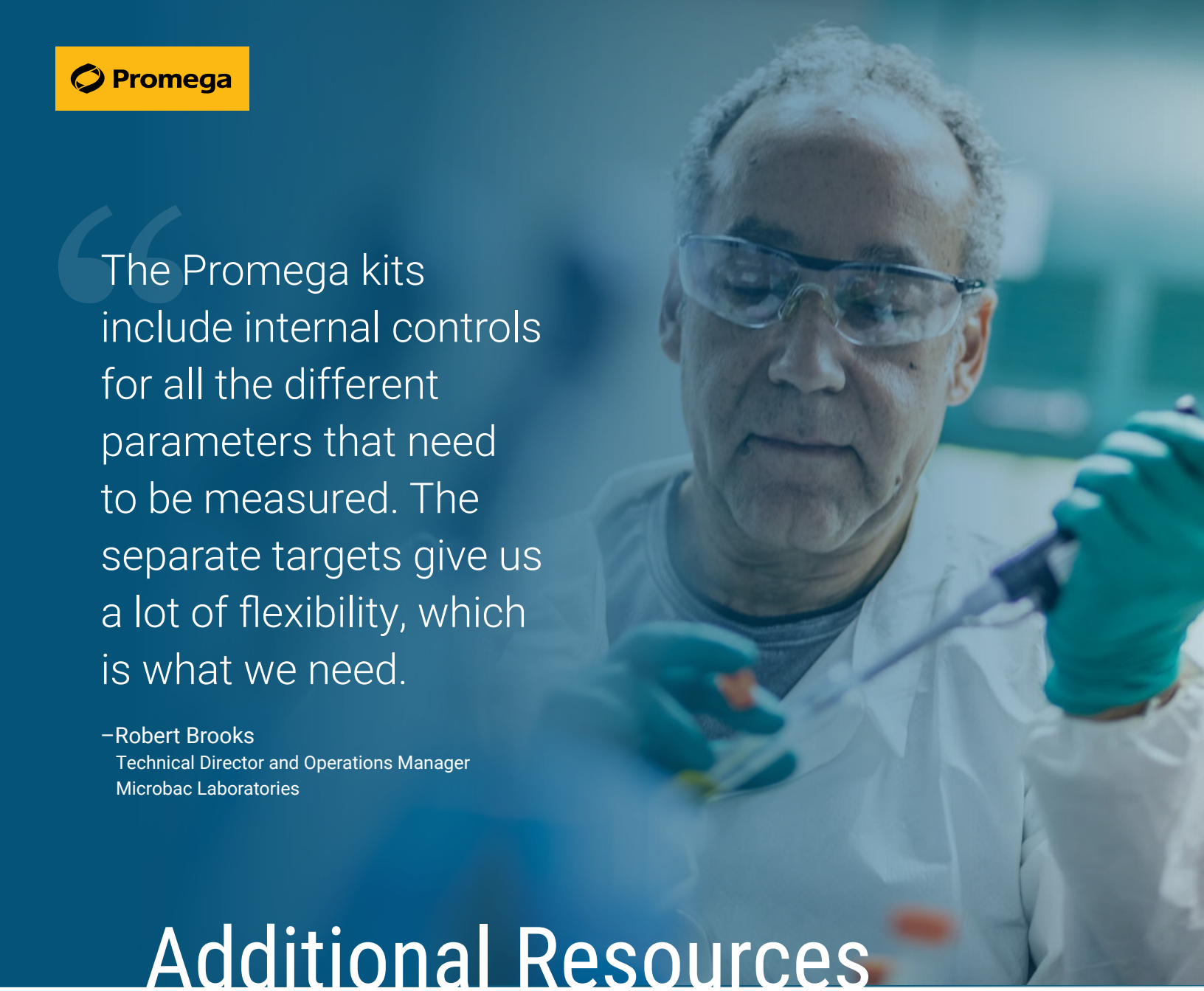
1. A direct capture method for purification and detection of viral nucleic acid enables epidemiological surveillance of SARS-CoV-2. Scientists conducted wastewater surveillance for COVID-19 in Dane County, Wisconsin, using a direct capture workflow.

2. How a new Wastewater Surveillance Method Predicted COVID-19 Cases in Italy. This blog post discusses how the Promega PCR-based workflow was applied in Italy to predict cases of COVID-19.

3. Longitudinal monitoring of SARS-CoV-2 in wastewater using viral genetic markers and the estimation of unconfirmed COVID-19 cases. Wastewater surveillance in northern Nevada demonstrated its utility in early detection of COVID-19.

5. Evaluation of Methods and Processes for Robust Monitoring of SARS-CoV-2 in Wastewater. Researchers optimized a wastewater analysis workflow using Promega Wizard® and Maxwell® RSC Enviro Kits.

4. Evaluation of viral concentration and extraction methods for SARS-CoV-2 recovery from wastewater using droplet digital and quantitative RT-PCR. Researchers evaluated five different concentration methods and four kits for nucleic acid extraction in wastewater analysis, including those offered by Promega.



> The Promega kits include internal controls for all the different parameters that need to be measured. The separate targets give us a lot of flexibility, which is what we need.
>
> –Robert Brooks
> Technical Director and Operations Manager
> Microbac Laboratories

Additional Resources

Have questions about wastewater testing tools and resources?

Ask an expert:
applied@promega.com

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