Wastewater-based epidemiology workflows with QIAcuity® digital PCR

Colin Donohoe, Domenica Martorana, Oezlem Karalay, Ronny Kellner, Dominic O'Neil, Tanya Sperling, Andreas Hecker, Daniel Heinz Löfgren, Andreas Missel, Francesca Di Pasquale

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

QIAcuity digital PCR is ideally suited for wastewater-based epidemiology

Wastewater-based epidemiology has been established as a powerful tool for tracking the spread of viruses such as SARS-CoV-2 within large populations. However, sensitively detecting trace amounts of viruses in wastewater samples comes with significant challenges. The physical and chemical properties of wastewater make nucleic acid extraction difficult, while PCR inhibitors inherent to environmental samples can interfere with PCR detection. Therefore, a carefully considered workflow is crucial to maximizing the sensitivity and accuracy of viral detection in wastewater with PCR. Here we demonstrate that QIAcuity digital PCR (dPCR) paired with extraction technologies from QIAGEN dedicated for environmental samples is an ideal combination for detecting SARS-CoV-2 in wastewater.

Compared with qPCR, QIAcuity dPCR does not require a prequalified standard curve for quantification. QIAcuity dPCR is also less sensitive to PCR inhibitors from environmental samples than qPCR. Additionally, dPCR chemistries tailored to accommodate environmental samples, such as the QIAcuity OneStep Advanced Probe Kit, can bolster PCR sensitivity even further.

Viruses like SARS-CoV-2 are enriched in wastewater solids compared to the aqueous phase. However, nucleic acids extracted from wastewater solids using standard methods strongly inhibit PCR and dramatically reduce workflow sensitivity. In contrast, QIAGEN sample extraction technologies containing proprietary Inhibitor Removal Technology® (IRT) increase workflow sensitivity by letting researchers confidently interrogate wastewater solids without the concern of PCR inhibition.

The QIAcuity workflow described here can also be extended to further targets, such as influenza, norovirus, monkeypox and polio, among others, providing a powerful tool for understanding the prevalence and spread of these pathogens in the environment.

Why combine QIAcuity digital PCR and wastewater for use in epidemiology?

Cost

- Worldwide wastewater monitoring could save up to 1 billion USD for national monitoring programs depending on frequency of sampling and population

Sensitivity

- One infected individual among 10,000 persons could potentially be detected

Coverage

- Everyone 'opts in' for testing
- 2.1 billion people could be monitored globally in 105,600 sewage treatment plants

Precise and absolute quantification

- QIAcuity digital PCR allows for end-point detection as in conventional PCR
- A standard curve is not required – no need to rely on the correctness of the concentration of the standard
- Higher precision than qPCR made possible by the thousands of partitions interrogated in a single reaction

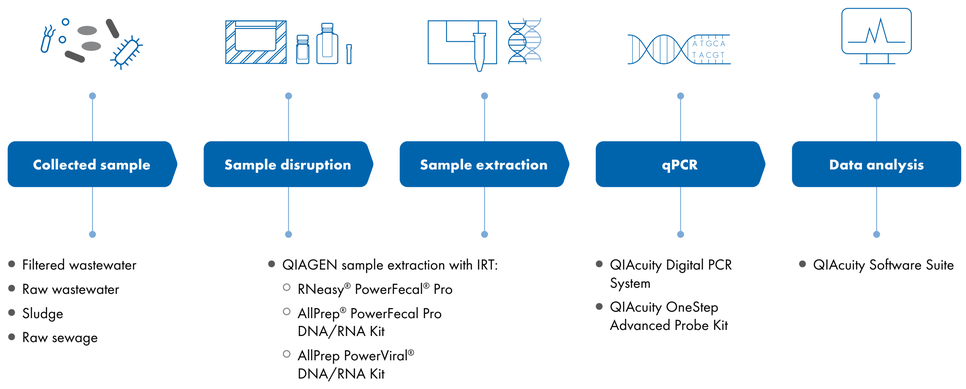
Robust

- Increased reproducibility and reduced variability leads to better inter-laboratory comparability

Resistant to inhibitors

- Higher robustness for viral detection from complex samples – wastewater samples are diverse and contain high levels of PCR inhibitors

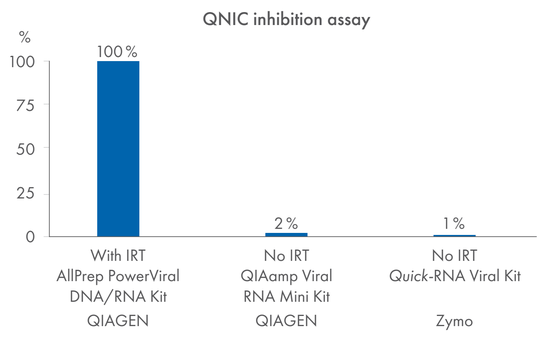
QIAGEN sample to insight workflow for wastewater-based epidemiology with QIAcuity dPCR



QIAcuity dPCR wastewater workflow.

When using wastewater solids as input material, employing sample extraction methods with inhibitor removal technology (IRT) is essential

- Publications have shown that enveloped viruses such as SARS-CoV-2 are enriched in the solid fraction of wastewater (1, 2).
- However, the solid fractions of wastewater harbor significant levels of RT-dPCR inhibitors.
- Without the use of IRT in sample extraction (e.g., QIAGEN QIAamp® Viral RNA Mini Kit, Zymo *Quick*-RNA® Viral Kit), eluates from wastewater solids are severely inhibitory to RT-dPCR when compared to samples extracted with kits that use IRT – such as the QIAGEN AllPrep PowerViral DNA/RNA Kit.



Relative quantification of a spike-in RNA target (QNIC) added to QIAcuity RT-dPCR reactions shows severe inhibition in waste-water samples extracted without IRT.

QIAGEN RT-dPCR workflow demonstrates high sensitivity in detecting SARS-CoV-2 in wastewater

RNA extraction

Two wastewater treatment facilities tested (H and M) using 40 mL wastewater for each extraction. RNA was extracted from:

- QIAGEN: Pelleted solids – RNeasy PowerFecal Pro Kit
- Bio-Rad: Clarified supernatant – Zymo *Quick*-RNA Viral Kit (Bio-Rad recommended kit)

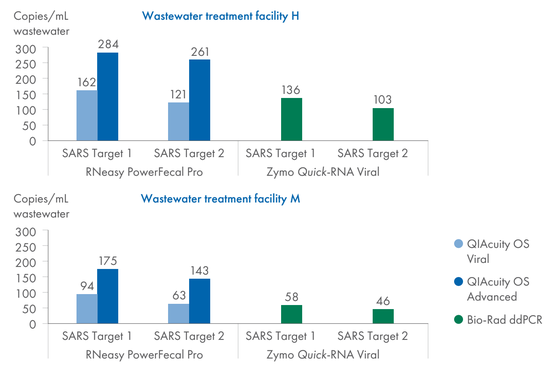
QIAGEN dPCR

Eluates (9 µL) were assayed in a 40 µL reaction in 26k Nanoplates using the following dPCR chemistries:

- QIAcuity OneStep Viral + GT Molecular Assays
  - GT Molecular SARS Assays (N2 FAM™, N1 ROX™) with GT Molecular cycling
- QIAcuity OneStep Advanced + GT Molecular Assays
  - Enhanced chemistry to better tolerate PCR inhibitors found in environmental samples
  - No Enhancer GC used
  - GT Molecular SARS Assays (N2 FAM, N1 ROX) with GT Molecular cycling

Bio-Rad ddPCR

- PREvalence™ SARS-CoV-2 Wastewater Kit
- Eluates (9 µL) were assayed in 20 µL Bio-Rad ddPCR reaction (recommended protocol)
- Bio-Rad SARS Assays (N2: FAM, E: HEX™)



Comparison data obtained through experiments conducted by QIAGEN R&D in Hilden, Germany.

Summary

QIAGEN offers an end-to-end, sample-to-insight workflow for wastewater-based epidemiology (WWBE) that addresses two critical aspects for success:

Sample extraction

- Wastewater solids are enriched for viruses of interest, but they contain high levels of RT-dPCR inhibitors
- Extraction methods with inhibitor removal technology (IRT) like those from QIAGEN are key to unlocking the potential of wastewater solids in WWBE

RT-dPCR chemistry

- The choice of RT-dPCR chemistry is also key to maximizing sensitivity.
- Compared with the QIAcuity OneStep Viral RT-PCR Kit, the QIAcuity OneStep Advanced Probe Kit is designed to tolerate inhibitors found in wastewater

Reference:

1. Kim S, et al. SARS-CoV-2 RNA is enriched by orders of magnitude in primary settled solids relative to liquid wastewater at publicly owned treatment works. *Environ Sci (Camb).* 2022;8(4):757-770.
2. Ye Y, Ellenberg RM, Graham KE, Wigginton KR. Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater. *Environ Sci Technol.* 2016;50(10):5077-5085.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

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Sample to Insight