APPLICATION NOTE

Detecting and Quantifying Pathogens in an Environmental Setting with Genomic Tools that Scale

Using the Biomark X9 System to accelerate data to decisions with broad pathogen coverage in wastewater and antimicrobial resistance

Introduction

Accurate pathogen detection and surveillance are critical for public health preparedness, especially with rising global threats such as emerging infectious diseases and antimicrobial resistance. Despite advancements in technology, conventional testing methods continue to face significant limitations, including:

- Limited throughput: Conventional qPCR platforms often struggle to process large sample volumes or detect multiple pathogens in a single run
- Slow turnaround time: Delayed results impede timely outbreak responses and decision-making
- High manual effort: Manual workflows increase the risk of error and add to labor costs

These challenges highlight the need for an innovative solution capable of delivering rapid, reliable and scalable pathogen detection.

The Biomark™ X9 System for High-Throughput Genomics redefines high-throughput pathogen detection with features specifically designed to overcome the limitations of traditional platforms, providing:

- **Speed to insights:** The Biomark X9 System generates actionable data faster, accelerating outbreak prevention and response
- **Automated efficiency:** With a single run, walk-away workflows reduce technician time and minimize errors
- **Unmatched scalability:** Simultaneous detection and quantification of up to 96 pathogens across 96 samples in one run significantly increases throughput

This application note outlines case studies using high-throughput pathogen detection in three different settings:

Case study 1: Wastewater surveillance for outbreak prevention

Researchers applied high-throughput qPCR to simultaneously detect 22 pathogens in a single run for rapid detection of pathogens when monitoring community health.

Case study 2: Tracking antibiotic resistance in environmental samples

Using qPCR, researchers simultaneously quantified pathogens and ARGs in one run, identifying critical links in the spread of resistance and addressing the need to monitor co-existence of bacterial pathogens and ARGs in wastewater.

Setting a new standard in automated genomic workflows, the Biomark X9 System offers the unique ability to produce high-impact data 2x faster than other systems, with higher pathogen coverage detected per run

Genomics using the Biomark X9 System: Significantly lower hands-on time and more datapoints per run deliver much higher labor efficiency

More than 12x the data, with half the hands-on time

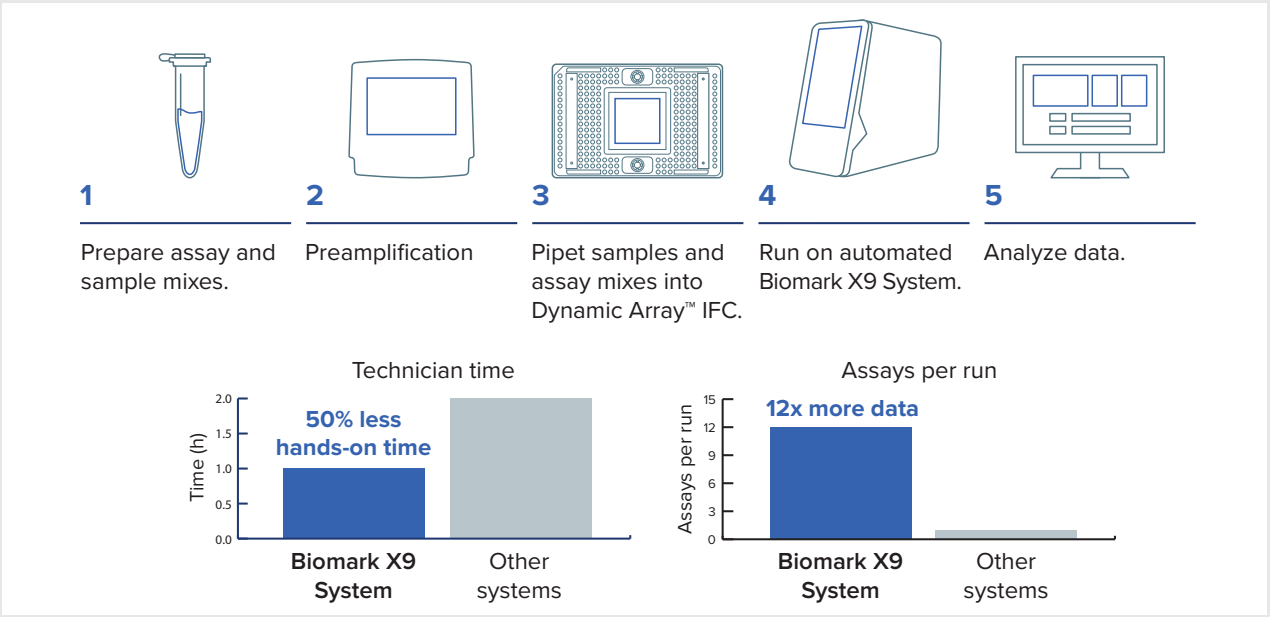

Figure 1. The Biomark X9 System simplifies genotyping and gene expression workflows. The significant increase in data generation can be crucial when results need to be analyzed quickly[1,2]. The Biomark X9 System expands the number of samples that can be processed in the same amount of time, with reduced technician effort, as demonstrated in the case studies below.

By leveraging workflows that can generate data quickly as well as analyze multiple pathogens of interest simultaneously, the Biomark X9 System empowers researchers to get the actionable information they need for faster turnaround and more informed decision-making. The ability to scale the number of samples per run and the number of assays per sample also increases throughput, maximizes detection, minimizes consumables consumption and reduces both variability and the risk of assay interference per singleplex simplicity.

The Biomark X9 System provides a high-quality automated workflow that can test the most samples against up to 96 assays in a single run.

CASE STUDY

Simultaneous detection and quantification of multiple pathogens in wastewater for faster response times

The ability to rapidly detect and monitor emerging pathogens and their variants by regular pathogen surveillance is essential for effective outbreak prevention and response management. Researchers from the University of Yamanashi in Japan applied qPCR using the Biomark system to simultaneously detect SARS-CoV-2, several SARS-CoV-2 variants and other pathogenic viruses in wastewater[1].

The study demonstrated that the Biomark system:

- Enabled testing of multiple assays for rapid pathogen detection in wastewater for community monitoring in Japan
- Successfully and reproducibly detected all targeted variants within wastewater samples
- Highlighted that high-throughput qPCR is a time- and cost-efficient method for tracking COVID-19 and broadly monitoring community health
- **Provided speed to insights by running 22 assays in one run versus conventional testing**

High-throughput qPCR simultaneously tested 22 different assays, including SARS-CoV-2, nucleotide substitutions and other pathogenic viruses for rapid results

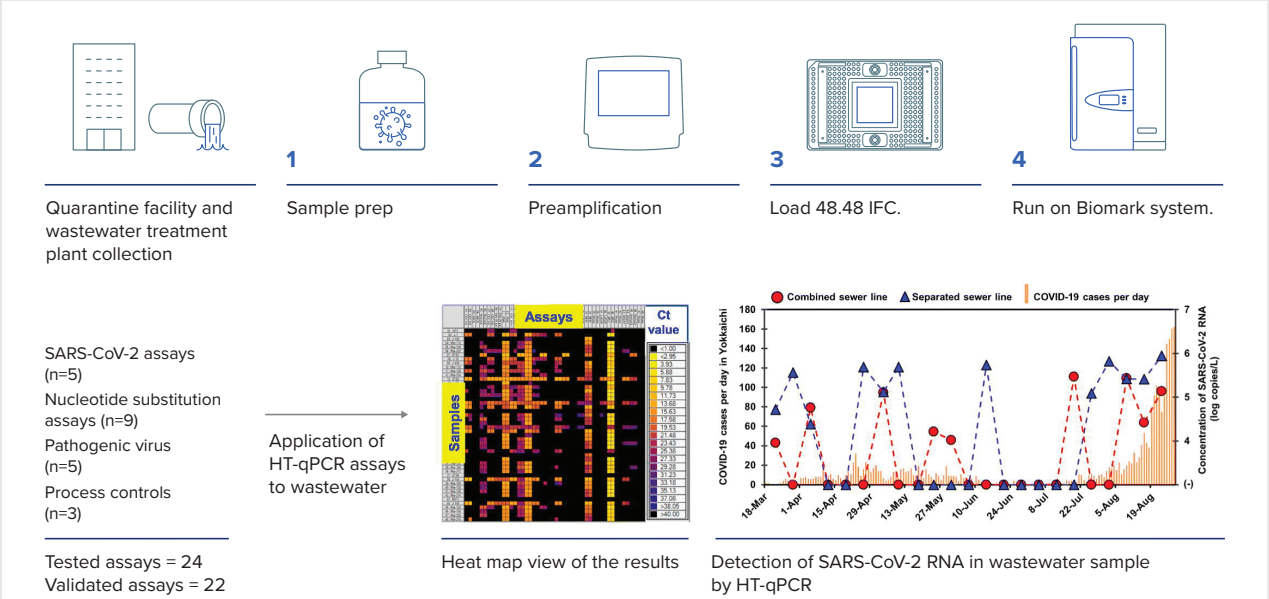

Figure 2. Researchers detected more pathogens with the simultaneous multiplexing of assays, which serves as a rapid method for efficient and cost-effective pathogen surveillance, enabled by Biomark systems.

Malla, B. et al. "Application of a high-throughput quantitative PCR system for simultaneous monitoring of SARS-CoV-2 variants and other pathogenic viruses in wastewater." *Science of the Total Environment* 853 (2022): 158659.

CASE STUDY

Uniquely assessing different assay types in one run: Tracking antibiotic resistance genes and various pathogens using the Biomark X9 System

Knowledge of the link between pathogens and antibiotic resistance is important because the co-existence of bacterial pathogens and antibiotic resistance genes (ARGs) increases the spread of antibiotic resistance ability of susceptible bacteria. Researchers at the University of Yamanishi in Japan[2] simultaneously quantified a set of ARGs and pathogens in wastewater using high-throughput qPCR to better understand the relationship between pathogens and antibiotic resistance in environmental waters.

The Biomark X9 System enabled addition of multiple pathogens and resistance genes in the same run without requiring reflex testing.

Microfluidics-based high-throughput qPCR using the Biomark X9 System:

- Successfully detected a variety of pathogens and ARGs in a single run
- Offers a cost- and time-efficient solution for epidemiological surveillance, enabling assays to be modified to rapidly adjust to emerging and re-emerging infectious diseases and ARGs
- **Highlights the capability of the Biomark X9 System to combine and investigate diverse groups of targets across many samples in a single run**

"The sole objective of investigating bacterial pathogens and ARGs together in this study is to highlight the capability of HT-qPCR technology to combine and investigate diverse groups of targets in a single run[2]."

Shrestha, S. et al. "High-throughput microfluidic quantitative PCR system for the simultaneous detection of antibiotic resistance genes and bacterial and viral pathogens in wastewater." *Environmental Research* 255 (2024): 119156.

Comprehensive analysis of 14 distinct assays, covering multiple bacterial and viral pathogens alongside ARGs in a single run

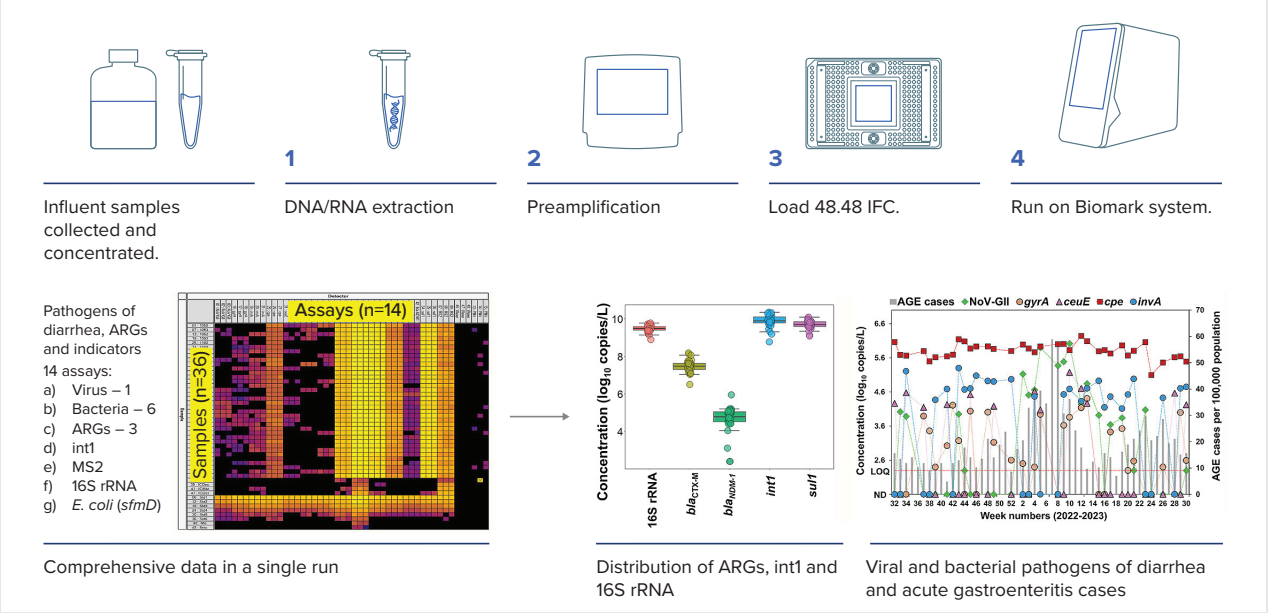

Figure 3. A single 48.48 integrated fluidic circuit (IFC) permits 2,304 reactions with 10 nL per chamber, reducing costs, time, labor and reagent requirements. This high-throughput, high-coverage capability is important since wastewater harbors diverse antibiotic-resistant bacteria and ARGs from human, animal and environmental sources.

Impact that matters

These studies demonstrate that the Biomark platform uniquely enables meaningful scientific impact through the rapid and simultaneous detection of multiple targets in one run with half the hands-on time. Each run can generate up to 12x more data for greater insights into possible disease outbreaks or epidemiological surveillance.

Summary

- High-throughput qPCR using the Biomark X9 System enables small volumes of samples to be effectively tested and allows for the simultaneous detection and quantification of a large number of pathogens in a single run
- This is demonstrated in case studies using the Biomark system:
  - To simultaneously test many different types of assays in one run for faster insights
  - For comprehensive analysis of multiple pathogens and ARGs with the capability to easily add more content without needing reflex testing

References

1. Malla, B. et al. "Application of a high-throughput quantitative PCR system for simultaneous monitoring of SARS-CoV-2 variants and other pathogenic viruses in wastewater." *Science of the Total Environment* 853 (2022): 158659.
2. Shrestha, S. et al. "High-throughput microfluidic quantitative PCR system for the simultaneous detection of antibiotic resistance genes and bacterial and viral pathogens in wastewater." *Environmental Research* 255 (2024): 119156.

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LAB-00008 Rev 01