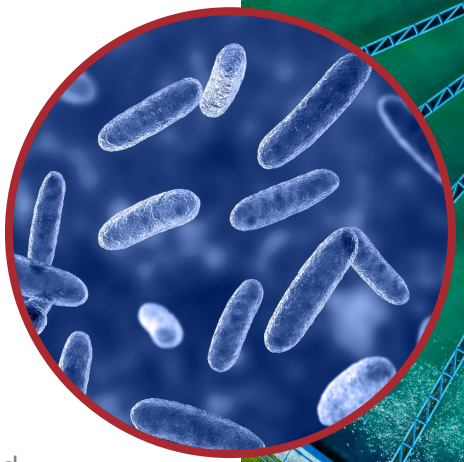
CASE STUDIES

Revolutionizing Wastewater Surveillance and Pathogen Identification With the Latest Microfluidics Technology



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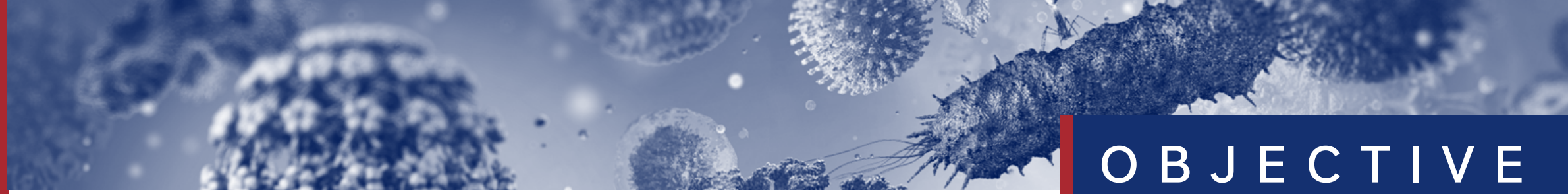
WASTEWATER CASE STUDIES

CONTENT

STUDY 1



OBJECTIVE

High-throughput microfluidic quantitative PCR system for the simultaneous detection of antibiotic resistance genes and bacterial and viral pathogens in wastewater

Shrestha, S. et al. *Environmental Research* (2024)

Simultaneously quantify antibiotic resistance genes (ARGs) in wastewater using high-throughput qPCR to understand the relationship between pathogens and antibiotic resistance in environmental waters

KEY TAKEAWAYS

- Microfluidics-based high-throughput qPCR successfully analyzed pathogens and ARGs in a single run.
- Norovirus genogroup II was linked to cases of acute gastroenteritis but bacterial pathogens, while detected in many samples, were not.
- ARGs (*sul1, blaCTX-M* and *blaNDM-1*) were prevalent, but further study on applicability as an indicator of overall resistance must be performed.

Utilization of Standard BioTools™ products

- Biomark system
- 48.48 Dynamic Array™ IFCs (integrated fluidic circuits)
- Standard BioTools Real-Time PCR Analysis Software

Learn more about the Biomark™ X9 System for High-Throughput Genomics >

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.

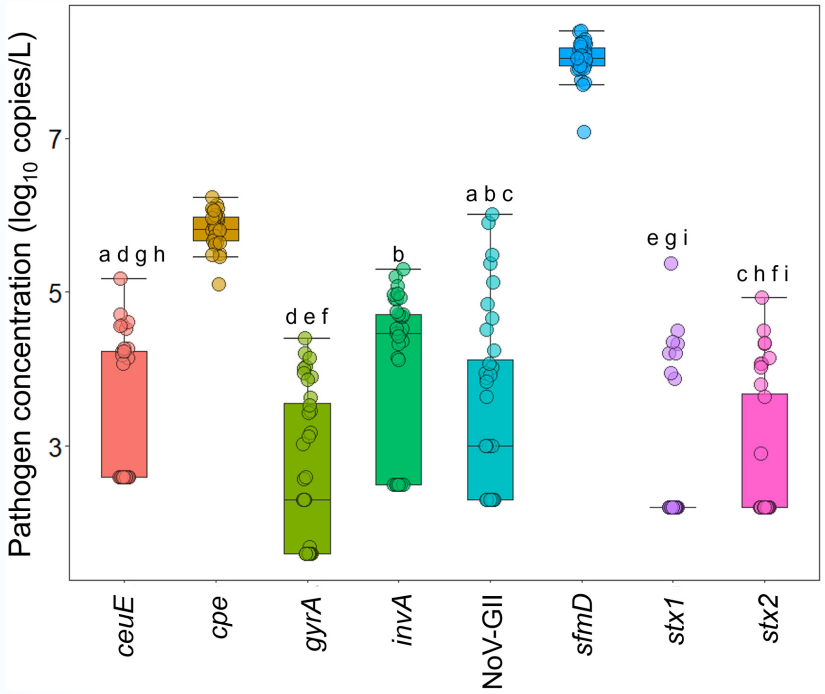
BACKGROUND

- Reliance on ill individuals seeking testing as surveillance for emerging pathogens leads to underreporting and delays in managing the spread of foodborne illnesses.
- ARGs are key in spreading resistance among environmental microbes and human pathogens.
- Wastewater-based epidemiology focuses on identifying and quantifying pathogens and biological markers present in wastewater and is vital to informing public health efforts.

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.

RESULTS

- All qPCR assays exhibited high performance with R2 ≥0.978 and efficiencies ranging from 90.5% to 117.7%.
- Fifty-six percent of samples were positive for norovirus genogroup II, 67% were positive for *Salmonella* spp. and *Campylobacter jejuni* and 36% were positive for *Campylobacter coli*. Norovirus detection rates and concentrations were higher in winter and spring.
- All samples tested positive for ARGs, irrespective of season.
- Insights from this study suggest the importance of a high-throughput qPCR system for wastewater-based epidemiology studies.



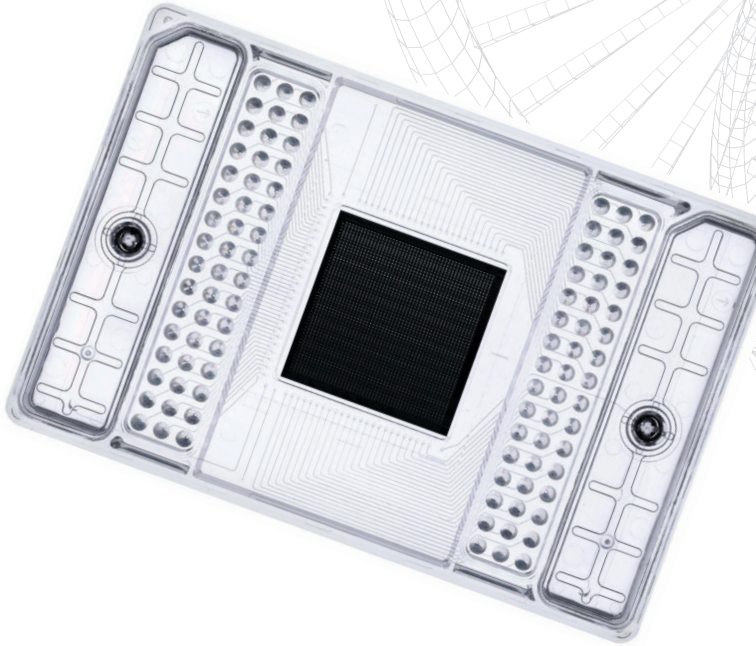
Box plot of concentration distribution of bacterial and viral pathogens and *E. coli* in wastewater samples

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.

STUDY OVERVIEW

- Culture-based methods are standard for wastewater analysis, but culture-independent techniques, such as qPCR, offer a more labor-efficient and comprehensive approach to capturing ARGs in wastewater.
- A single 48.48 IFC permits 2,304 reactions with 10.1 nL per chamber, reducing costs, time, labor and reagent requirements.
- Thirty-six grab wastewater samples were collected from a wastewater treatment plant in Japan three times a month between August 2022 and July 2023. Samples were centrifuged, after which nucleic acid extraction, reverse transcription and qPCR were performed. Fourteen targets were included.

Learn more about IFCs >



48.48 Dynamic Array IFC

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.

STUDY 2

OBJECTIVE

Application of a high-throughput quantitative PCR system for simultaneous monitoring of SARS-CoV-2 variants and other pathogenic viruses in wastewater

Malla, B. et al. *Science of the Total Environment* (2022)

Simultaneously detect 22 targets including SARS-CoV-2, key variants and epidemiologically relevant viruses such as InfA, NoV-G1, NoV-GII, EnV, RVA and Aiv-1

KEY TAKEAWAYS

- A single gene target panel can be run using Standard BioTools Dynamic Array IFCs to assess multiple viruses in wastewater.
- Standard BioTools Dynamic Array IFCs provide satisfactory limit of detection (LoD) and throughput for monitoring the health of large populations via wastewater.

Utilization of Standard BioTools products

- Biomark HD system
- IFC Controller MX
- 48.48 Dynamic Array IFCs
- Standard BioTools Real-Time PCR Analysis Software

Learn more about the Biomark X9 System* >

* The Biomark X9™ System is the next-generation system of Biomark technology.

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): 158658.

BACKGROUND

- SARS-CoV-2 and its variants present a worldwide health concern.
- Rapid surveillance and detection of SARS-CoV-2, its variants and other pathogens are crucial to controlling spread.
- One method for quick detection of COVID-19 in large communities is monitoring wastewater runoff.

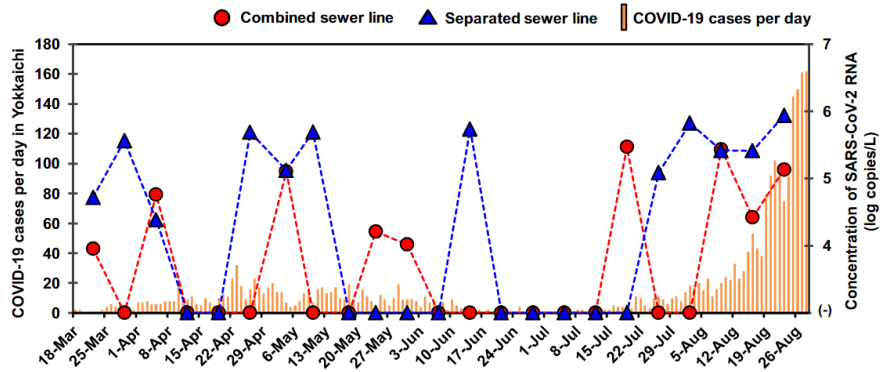
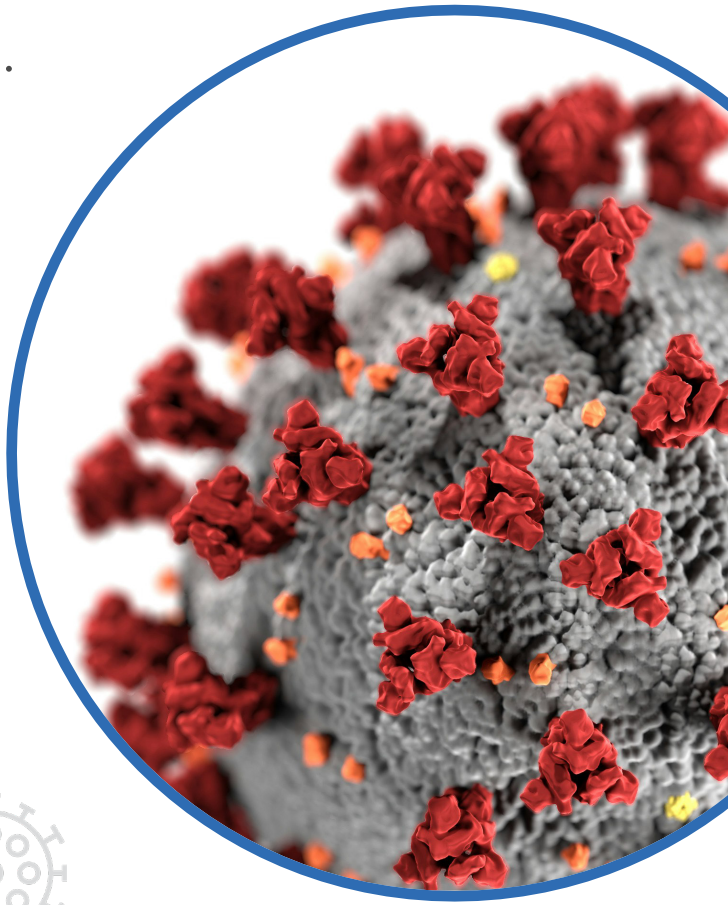


Fig. 1. Number of daily new reported cases of COVID-19 and the concentrations of SARS-CoV-2 RNA in influent samples of combined (denoted by solid circle) and separated sewer lines (denoted by solid triangle).



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): 158658.

RESULTS

- Standard BioTools IFCs were used because of demonstrated low LoD, their ability to run assays in parallel and include process controls and their low use of reagents (particularly important during periods of high testing and reagent shortages).
- Five targets were included for SARS-CoV-2 for increased sensitivity, and nine SARS-CoV-2 targets were included for variants of concern and emerging strains.
- This panel can easily be altered for other pathogens and can detect other viruses such as InfA, NoV-G1, NoV-GII, EnV and Aiv-1.

Detection of SARS-CoV-2 and SARS-CoV-2 nucleotide substituted RNA in a COVID-19 quarantine facility by HT-qPCR.

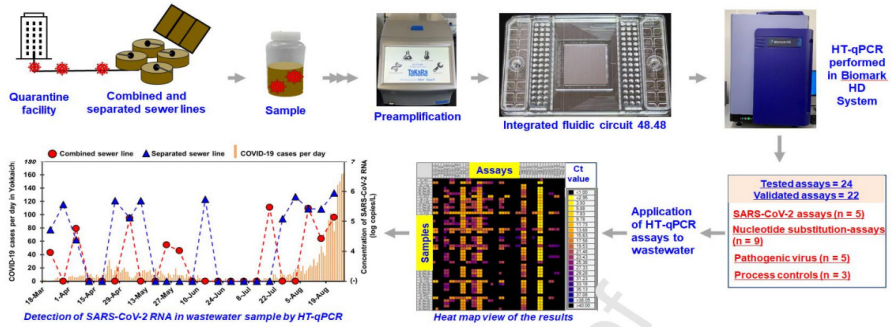
Date of sample collection (dd/mm/yyyy)	SARS-CoV-2 RNA (log copies/L)					SARS-CoV-2 nucleotide substituted RNA (log copies/L)			
	CDC N1	CDC N2	CDC N1 + N2	NIID	N_Sarbeco	E484K	L452R	N501Y	S69-70 del
27/10/2020	6.7	6.2	6.3	6.3	6.5	ND	ND	ND	ND
27/11/2020	7.6	7.0	7.2	7.0	7.0	ND	ND	ND	ND
24/12/2020	8.5	7.8	8.0	7.8	7.8	ND	ND	7.1	7.1
21/2/2021	6.0	5.3	5.7	5.1	5.6	ND	ND	ND	ND
No. of positive samples (%)	4 (100 %)	4 (100 %)	4 (100 %)	4 (100 %)	4 (100 %)	0 (0 %)	0 (0 %)	1 (25 %)	1 (25 %)
Mean ± SD[a] (log copies/L)	7.2 ± 1.1	6.6 ± 1.0	6.8 ± 1.0	6.6 ± 1.1	6.7 ± 0.9	NA	NA	7.1	7.1

SD, standard deviation; ND, not detected; NA, not applicable.
[a] Mean concentration of positive samples.

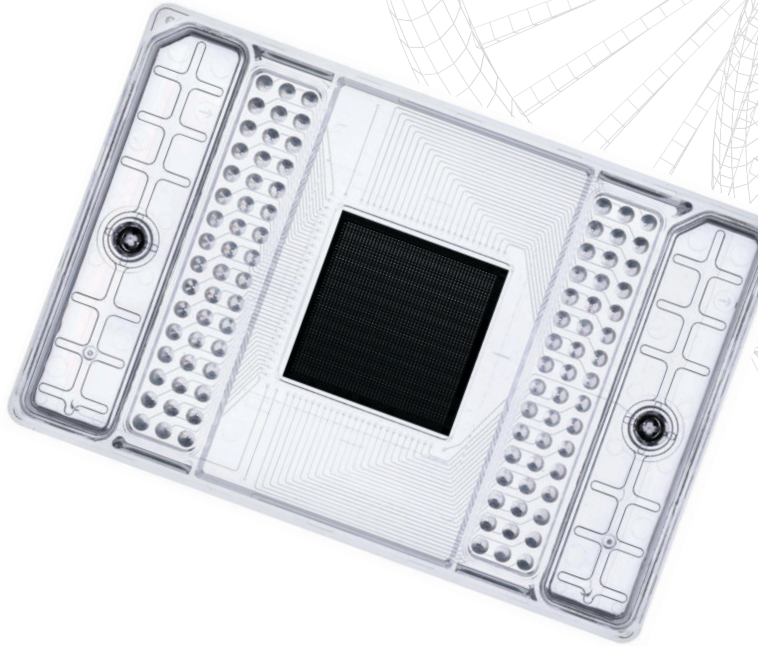
Malla, B. et al. "Application of a high-throughput quantitative PCR system for simultaneous monitoring of SARS-CoV-2 variants and other pathogenic viruses in wastewater." *Science of the Total Environment* 853 (2022): 158658.

STUDY OVERVIEW

- Samples were collected from different sewer lines coming from a SARS-CoV-2 quarantine facility.
- A 48.48 IFC was used with a preamplification step.
- A custom panel identified COVID-19 and other pathogen-positive samples.
- Process controls and relative standard curves were included to identify concentrations.



Learn more about IFCs >



48.48 Dynamic Array IFC

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): 158658.

STUDY 3

OBJECTIVE

Hospital discharges in urban sanitation systems: long-term monitoring of wastewater resistome and microbiota in relationship to their eco-exposome

Buelow, E. et al. *Water Research X* (2020)

Develop an approach to assess wastewater for antimicrobial resistance genes (ARGs) and microbial communities

KEY TAKEAWAYS

- Hospital and urban wastewater ARGs remained stable over time.
- Hospital wastewater contains significantly higher loads of ARGs and antimicrobial resistance (AMR).
- AMR data shows no risk of increased spread when processing mixed wastewater samples.

Utilization of Standard BioTools products

- Biomark HD system
- 96.96 Dynamic Array IFCs
- Standard BioTools Real-Time PCR Analysis Software

Learn more about the Biomark X9 System* >

* The Biomark X9 System is the next-generation system of Biomark technology.

Buelow, E. et al. “Hospital discharges in urban sanitation systems: long-term monitoring of wastewater resistome and microbiota in relationship to their eco-exposome.” *Water Research X* 7 (2020): 100045.

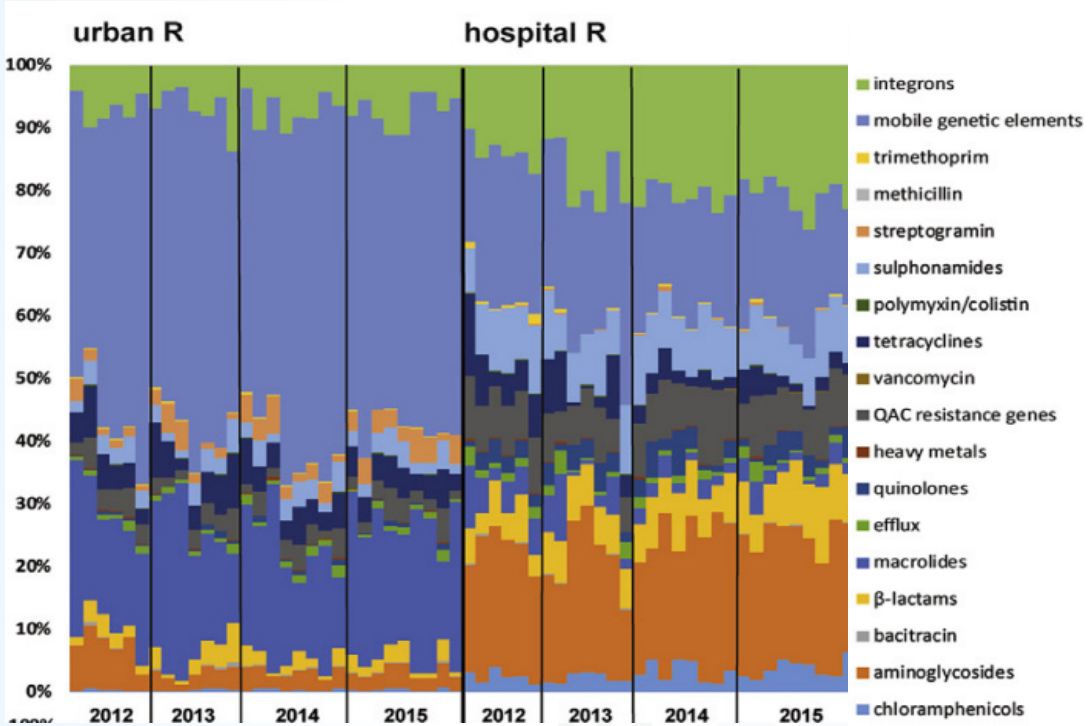
BACKGROUND

- Human interaction with the environment poses an important selective pressure for AMR.
- Urban and hospital wastewater contains a high diversity of ARGs. While wastewater treatment reduces the overall amount, ARGs still reach the environment.

Buelow, E. et al. “Hospital discharges in urban sanitation systems: long-term monitoring of wastewater resistome and microbiota in relationship to their eco-exposome.” *Water Research X* 7 (2020): 100045.

RESULTS

- The relative abundance of ARGs remained stable over time.
- A seasonal impact on individual resistance genes was seen in hospital wastewater.



Buelow, E. et al. “Hospital discharges in urban sanitation systems: long-term monitoring of wastewater resistome and microbiota in relationship to their eco-exposome.” *Water Research X* 7 (2020): 100045.

STUDY OVERVIEW

- DNA extraction was performed on recovered microorganisms filtered from water samples.
- Data was collected using a 96.96 IFC on the Biomark HD system.
- An 88-gene target panel was created that included genes related to resistance of the following categories:
  - Antibiotics
  - Quaternary ammonium compounds
  - Heavy metals
- Primer amplification efficiency, quality scores and cycle threshold were established.

Learn more about IFCs >

96.96 Dynamic Array IFC

Buelow, E. et al. “Hospital discharges in urban sanitation systems: long-term monitoring of wastewater resistome and microbiota in relationship to their eco-exposome.” *Water Research X* 7 (2020): 100045.

REVOLUTIONIZING WASTEWATER SURVEILLANCE AND IDENTIFICATION

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Learn more about these practical experiences and what our technology can achieve.

standardbio.com/area-of-interest/public-health

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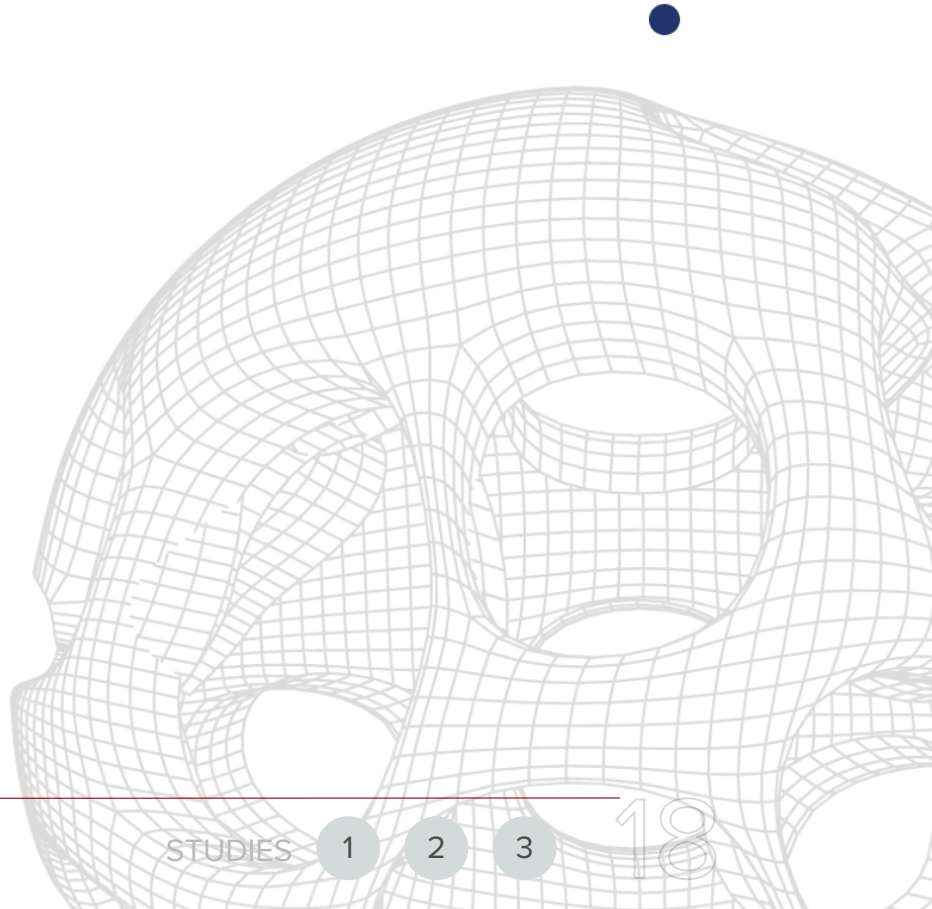

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