Title: Empowering Sewer Operations Teams with Rapid On-Site Contamination Monitoring

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Abstract:

The environmental management of sewage-related contamination in waterways remains a critical challenge, particularly during sewer-spill events or when ageing infrastructure fails. Traditional laboratory methods for detecting faecal indicator bacteria are often time-consuming and costly, delaying critical operational responses. This study presents the novel ZiP-P2 platform using the ZiP-Bactx-P2 assay for the rapid, field-deployable detection of human-specific *Bacteroides dorei*, a well-established proxy for sewage contamination for over 20 years.

The ZiP-P2 system delivers semi-quantitative results in under 20 minutes, with sensitivity comparable to PCR, all from a rugged, portable instrument suitable for remote field operations. This allows operational teams to conduct spatial and temporal sampling on site, rapidly guide source tracing and remediation decisions, and verify mitigation efficacy through digital reporting and geospatial mapping workflows.

Field trials demonstrated the platform's capacity to detect contamination events in near real time, drastically reducing turnaround from days to minutes. This capability significantly improves operational efficiency by enabling rapid, on-site decision-making, reducing reliance on laboratory testing and cutting associated costs by up to 90%. While laboratory testing still plays a role in regulatory confirmation, the ability to detect human-specific contamination quickly and at scale makes ZiP-P2 well suited for emergency sewer spill response, routine catchment risk assessments, and targeted investigations of failing infrastructure. Adoption of the ZiP-P2 system represents a significant step forward in water sector innovation, delivering actionable microbial data when and where it matters.

Introduction

Urban water catchments face multiple sources of faecal contamination, including stormwater, ageing sewer infrastructure, and on-site wastewater systems. This is especially concerning where urban waterways discharge into recreational areas, creating potential risks to public health. To manage these risks effectively, it is important to detect and map human-specific contamination.

Common field indicators such as ammonia or electrical conductivity (EC) are sometimes used to infer contamination, but they lack the specificity needed for confident decision-making. For example, ammonia may come from cleaning products rather than sewage, and EC is highly variable due to rainfall, sediments, or urban runoff. As a result, these measures can either overestimate or completely miss human-sourced faecal pollution.

This report evaluates *Bacteroides dorei*, a highly specific marker of human faecal input, as a rapid tool for identifying sewage contamination hotspots in urban catchments. Unlike

conventional indicators such as *E. coli*, this assay confirms faecal contamination of human origin, giving greater confidence in source tracking. To measure *B. dorei* on site we used the ZiP-Bactx-P2, a portable molecular test providing results in around 20 minutes, at a cost about 90% lower than laboratory testing. We examine how this approach enables more frequent sampling and rapid feedback, with substantial financial and environmental benefits for operators

Discussion

Catchment Assessments Using ZiP-P2

The ZiP-P2 system was evaluated across an urban catchment to detect human faecal contamination. By targeting *Bacteroides dorei*, a sewage marker, the tool provided rapid and reliable insights into potential sewage inputs at stormwater outfalls and creek outlets. Each assay produced a semi-quantitative result based on detection time, classified as: Non-detect (0), Positive-Low (1), Positive-Moderate (2), or Positive-High (3). To generate a consistent interpretation across sampling sites, results were combined into a colour-coded Contamination Index Eq. (1).

The index incorporated both detection strength (S) as a score from 0-3 for each result, and detection frequency (F), as a proportion of positive results out of total assays at a site.

The site contamination index (CI) was calculated as:

$$CI = (\sum S / N) \times F \tag{1}$$

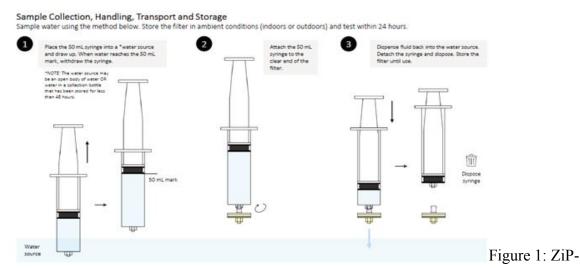
Where $\sum S$ is the sum of detection scores at a site, N is the total number of assays, and F is the frequency of positive results (positives/N). CI ranges from 0 (no detections) to 3 (all assays = high positives) (Table 1).

Table 1 Contamination Index (CI) score ranges and interpretation

| CI Score range | Contamination level | Signal strength | Detection frequency | Interpretation |
|----------------|---------------------|----------------------------|----------------------------|---|
| 0.00 | Non-detect | None | None | No human sewage signal detected across any sampling events. |
| 0.01 - 0.99 | Low | Weak | Infrequent to consistent | Weak signals across some or all samples; no strong detections observed. |
| 1.00 - 1.99 | Moderate | Moderate or mixed strength | Moderate to frequent | Moderate detections or a mix of low/moderate signals across multiple events. |
| 2.00 - 3.00 | High | Strong | Frequent to continuous | Strong and consistent signals (e.g. "High" or "Moderate" across most or all samples). |

Workflow

We developed and validated a quick and simple filtration-based sample preparation method for in-field use (Figure 1). Water samples were collected using a 60ml syringe or a sample bottle and transferred to a 60ml syringe. A filter was attached to the end of the syringe and the water is pushed through the filter and discarded. The filter was removed and either stored in a labelled zip lock bag for later use or used immediately.



Bactx-P2 Sample Preparation

We used a 5ml syringe and needle to draw test buffer solution (provided in the test) back through the filter and into the syringe. The needle was removed, and the buffer/sample mixture squirted back into the buffer tube, diluted and then transferred to the test cartridge and loaded into the ZiP-P2 test bay (Figure 2).

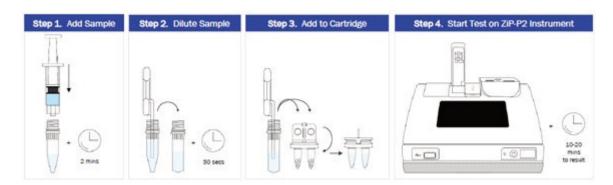


Figure 2: ZiP-Bactx-P2 workflow

Case Study 1: Balcombe Creek Transect

Objective

Assess human faecal contamination at stormwater outfalls along Balcombe Creek.

Sampling methodology

A total of 23 water samples were collected from six sites along Balcombe Creek covering a gradient from the upstream reference site (B6) to the estuary (B1). Sites B2 to B5 were positioned near or at the end of key stormwater outfalls. (Figure 3, Table 2). At each site, samples were collected at regular intervals over a 1-2- hour time-period. Results Below is a summary of the results (Figure 3):

• No Contamination Detected at Reference Site (B6):

Water quality at B6 showed no detectable contamination (CI=0), indicating that upstream sources were unlikely to be contributing to the issues observed further downstream at the time of sampling.

Weak, but consistent levels of Bacteroides detected at B2 indicate ongoing low level sewerage contamination (CI=0.88). This location would be considered moderate priority for further investigation

Results at sites B3, B4, and B5 indicated weak sewage signals with inconsistent detection patterns. These locations would be considered low priority for further investigation (Table 2).

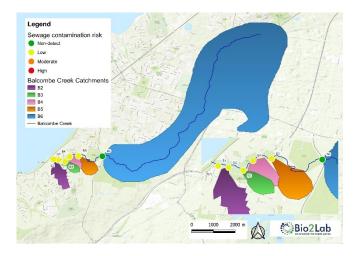


Figure 3 Map of sewage contamination risk along Balcombe Cree Operational Benefits of ZiP-P2

The ZiP-P2 system demonstrated clear advantages over traditional laboratory testing:

- Speed: Results were delivered in ~20 minutes compared with 4–5 days for lab analysis.
- Efficiency: Five samples can be processed per hour by one operator)
- Cost-effective: Average cost per test was ~\$65 compared to ~\$830 for lab-based Bacteroides testing and \$190.00 for *E. coli*. For daily sewer spill monitoring (~18

- samples), costs were lower using the Zip-P2 instrument than both laboratory based *E. coli* (27% cost reduction) and *Bacteroides* (90% cost reduction) (Table 2)
- Field-deployable: Samples were processed on-site, eliminating transport logistics and delays.

Table 2: Cost benefit analysis for the Zip-Bactx-P2 system, compared with traditional laboratory analysis for *B. dorei* and *E. coli*

| Parameter | B. dorei by ZiP-Bactx-P2 System | <i>B. dorei</i> by Laboratory qPCR # | E. coli by Laboratory MPN or ColilertTM # |
|--|------------------------------------|---|---|
| Test Cost | \$55.00 | \$700.00 | \$60.00 |
| Total Cost per Test (Incl. labour @ \$40/hr)* | \$65.00 | \$830.00 | \$190.00 |
| Daily cost for a sewer spill monitoring program (18 samples per day) Total Cost (Incl. Logistics and labour)* | \$1,170.00 | \$13,140.00 | \$1,620.00 |
| Turnaround Time (minimum) | 20-25 minutes | 96 hours (4 days) | 24 hours (1 days) |
| Result Availability | Instant | Delayed (lab-dependent) | Delayed (lab-dependent) |
| Setup/Transport Requirement | Minimal | Moderate-High | Moderate-High |

[#]Laboratory test costs estimated from Melbourne-based providers and industry sources.

Implications for Operators

The trials highlight ZiP-P2 as a practical, cost-effective tool for rapid sewage tracking and monitoring, enabling management actions to be rapidly prioritised based on real-time data. While laboratory testing still plays a role in regulatory confirmation, the ability to detect human-specific contamination quickly and at scale makes ZiP-P2 well suited for emergency sewer spill response, routine catchment risk assessments, and targeted investigations of failing infrastructure.

Overall, ZiP-P2 provides a rapid affordable option for identifying and addressing sewage contamination in complex urban environments, supporting faster decision-making and more efficient use of resources.

Conclusion

The ZiP2 monitoring system successfully detected and identified major sources of sewage contamination within catchments. By integrating spatial and temporal monitoring, it pinpointed critical discharge points and areas of elevated contamination, providing clear

^{*}Example of daily monitoring costs for a general sewer spill monitoring program. Assumes staff transport all 18 samples in one trip to a NATA accredited laboratory for analysis, (Industry sources).

evidence to guide prioritised management actions. The ZiP-P2 delivered same-day *B. dorei* results at ~\$65/test, a 90% cost saving compared to traditional lab methods (~\$830/test) with 4-5 day turnaround times. Even compared to *E. coli*, the industry standard, it was 27% more cost effective. With a throughput of five samples per hour, it also delivers significant efficiency gains for field operations. While confirmatory lab testing remains important for regulatory purposes, the ZiP-P2 provides a rapid, cost-effective tool for hotspot identification, sub-catchment prioritisation, and real-time microbial risk assessment. These findings support the ongoing use of rapid microbial detection tools, with future efforts focused on microbial source tracking during urgent events such as sewer spills. EPA Victoria has recently signalled approval for using the ZiP Bactx P2 assay during sewer spill operations, alongside *E. coli* testing once the spill was managed to ensure recreational suitability. Overall, the ZiP-P2 represents a scalable, proactive approach to water quality management in complex environments.

Acknowledgements

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References

Khodaparast, Meysam, Dave Sharley, Nickala Best, Stephen Marshall, and Travis Beddoe. "In-Field LAMP Assay for Rapid Detection of Human Faecal Contamination in Environmental Water." Environmental Science: Water Research & Technology 8, no. 11 (2022): 2641–51. https://doi.org/10.1039/D2EW00433J.

Khodaparast, Meysam, Dave Sharley, Stephen Marshall, and Travis Beddoe. "Advances in Point-of-Care and Molecular Techniques to Detect Waterborne Pathogens." Npj Clean Water 7, no. 1 (2024): 74. https://doi.org/10.1038/s41545-024-00368-9.

Khodaparast, Meysam, Dave Sharley, Stephen Marshall, and Travis Beddoe. "Rapid Detection of Viable Bacteroides in Sewage-Contaminated Water Using Sodium Dodecyl Sulfate and Propidium Monoazide Combined with Loop-Mediated Isothermal Amplification (LAMP) Assay." Environmental Science: Water Research & Technology 10, no. 4 (2024): 787–96. https://doi.org/10.1039/D3EW00585B.