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A Sustainable, End-to-End Workflow for Wastewater Pathogen Surveillance

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INTRODUCTION

Wastewater surveillance provides community-level insight into circulating pathogens, but implementation is challenging due to:

- Variability in pathogen shedding rates
- The dilution, decay and fragmentation of targets within sewers
- Chemical inhibitors that can reduce assay sensitivity
- Sample processing decisions (e.g. wastewater fraction chosen)
- Bioinformatic reference database and normalization biases, which can contribute to false positives or false negatives

Aim: Develop a sustainable, end-to-end wastewater surveillance workflow built on streamlined and standardized collection, flexible processing, scalable analysis and actionable reporting.

STEP 1: SAMPLE COLLECTION

Wastewater Stabilization Buffer (WSB)[1] is a sample collection buffer developed in partnership with the CDC. WSB:

- **Stabilizes** nucleic acids at room temperature for ~1 week
- **Inactivates pathogens** for safer sample transport (**Fig. 1**)
- Uses a color indicator to confirm pathogen inactivation
- **Concentrates nucleic acids**, removing the dependence on laborious ultrafiltration (**Fig. 2**) or PEG precipitation (**Fig. 3**)

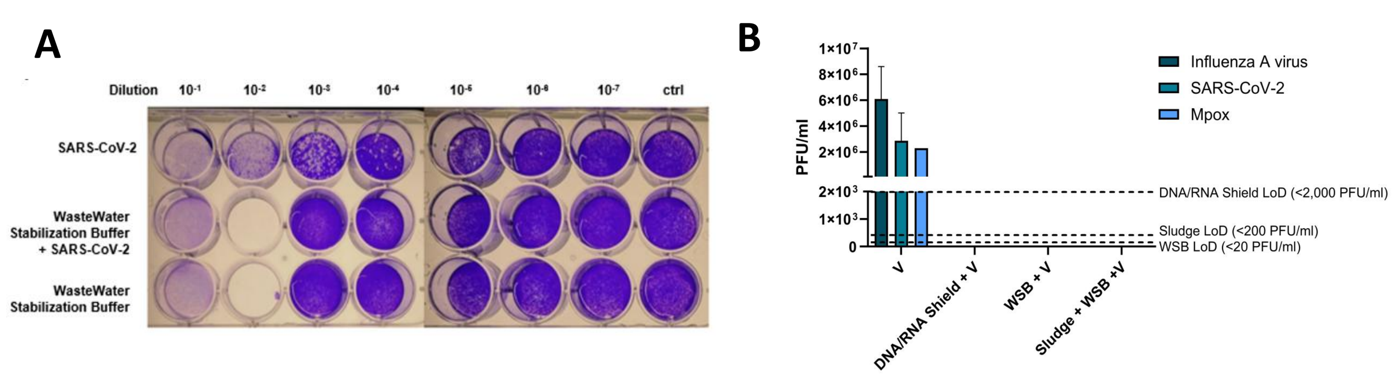


Fig. 1 Pathogen inactivation testing was conducted for enveloped viruses (SARS-CoV-2, influenza A, RSV and mpox), in two matrices: influent wastewater and domestic sludge. WSB viral inactivation assessed by plaque assay (A). WSB reduced viral titers by 3-5-fold, below the assay limit of detection (B).

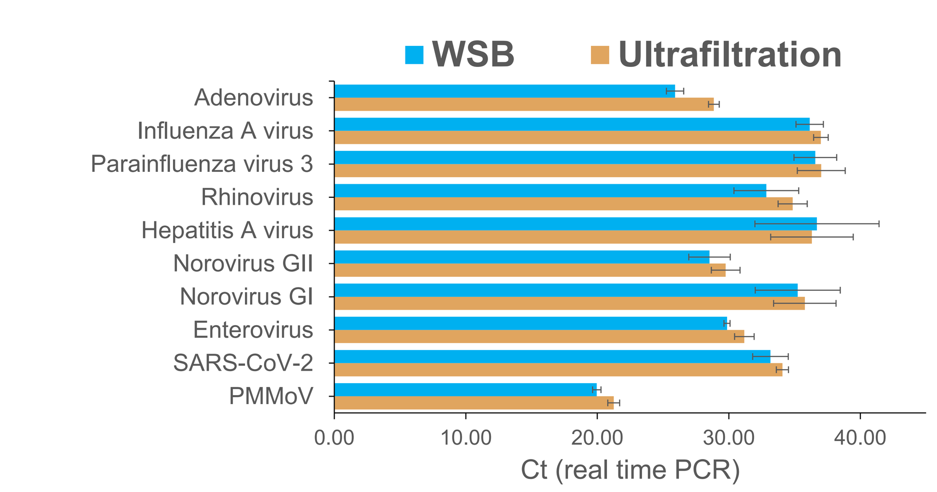


Fig. 2 Comparative standard real-time PCR analysis of viruses concentrated using WSB (blue) or vacuum ultrafiltration (orange).

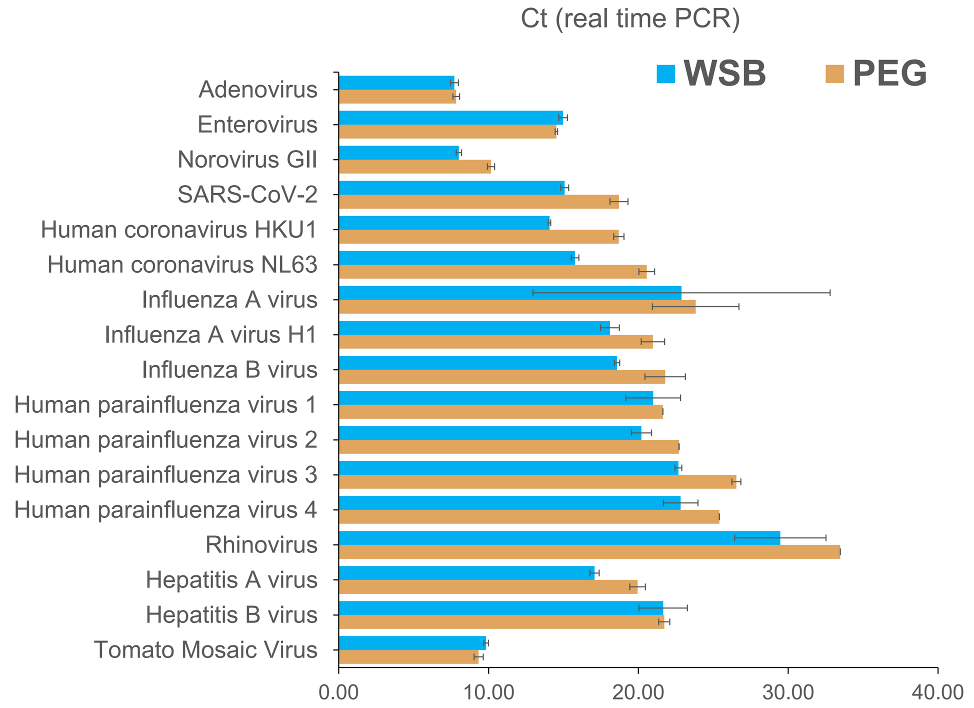


Fig. 3 Comparative microfluidics-based qPCR analysis[2] of viruses concentrated using WSB (blue) or PEG precipitation[3] (orange).

STEP 2: NUCLEIC ACID PURIFICATION

- Automated DNA/RNA co-purification workflow for dewatered solids, liquid or whole wastewater fractions using **Quick-DNA/RNA Viral MagBead Kit**[4]
- Bacterial **spike-in controls** used to monitor extraction efficiency.
- **Reduce inhibitors** and improve downstream amplification with the **OneStep PCR Inhibitor Removal column**[5] (**Fig. 4**)

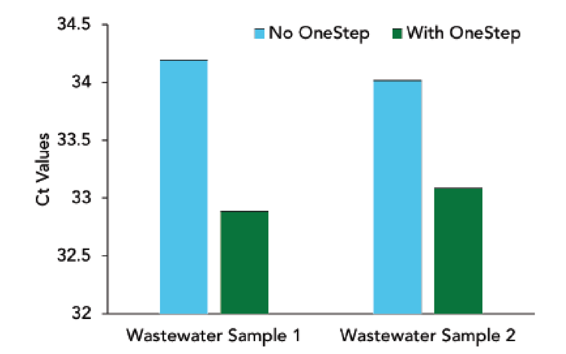


Fig. 4 RT-qPCR amplification of norovirus recovered from 40mL wastewater, untreated (blue) or treated with OneStep PCR Inhibitor Removal column (green).

STEP 3: PATHOGEN DETECTION

An optimal surveillance workflow depends on flexible assay design and assay choice depends on the objective (**Table 1**).

Table 1 Assay selection framework for molecular pathogen detection.

Assay	Primary Use	Strengths	Limitations
Digital/Microfluidic PCR	Rapid quantification	Digital PCR: "Gold standard" for wastewater, direct quantification, inhibitor tolerant, high sensitivity, fast turnaround Microfluidic PCR: high throughput, lower cost with additional targets, inhibitor tolerant, high sensitivity, fast turnaround	Digital PCR: higher cost with additional target, lower throughput, revalidation with additional targets Microfluidic PCR: indirect quantification
Probe Capture Sequencing	Targeted genomic surveillance	Broad multiplexing, genomic characterization, ~700 viral taxa covered	Inhibitor sensitivity, higher cost, longer turnaround restricted to predefined targets
Metagenomic Sequencing	Pathogen discovery	Unbiased detection of known and novel organisms	Lower sensitivity, higher sequencing requirements

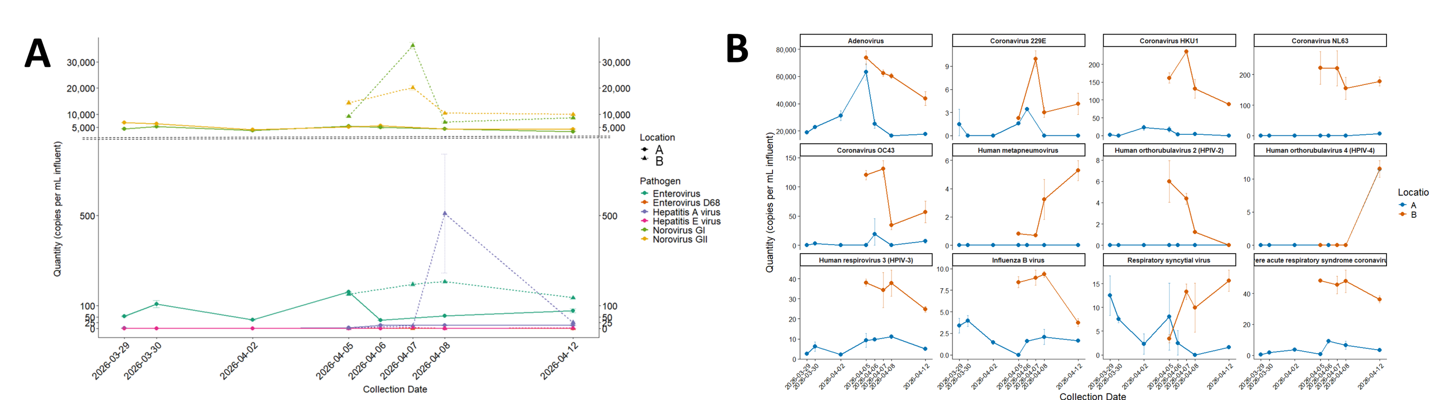


Fig. 5 Sensitive detection of >50 viral, bacterial, fungal and parasitic pathogens using microfluidics-based qPCR. Detection of selected enteric (A) or respiratory (B) viruses.

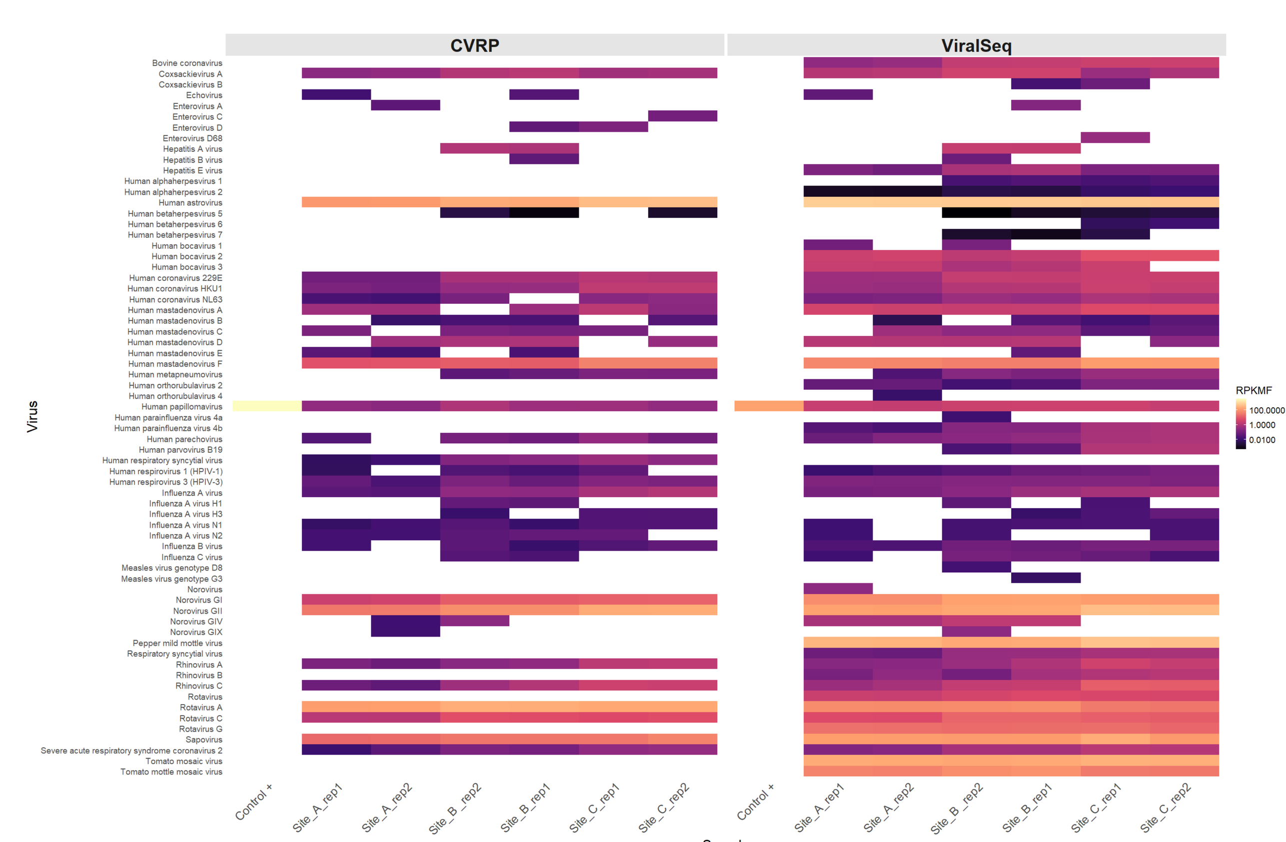
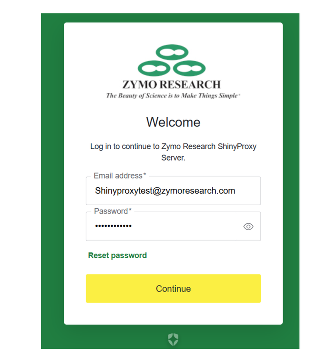


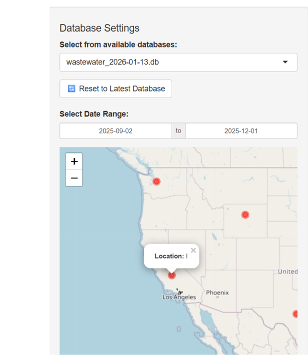
Fig. 6 Performance of customized probe capture sequencing panel, ViralSeq across three different sites. Tiles are colored according to reads per kilobase of reference genome per million reads passing filtering (RPKMF).

STEP 4: DATA INTERPRETATION

We developed a secure, web-based **data interpretation dashboard** for rapid, user-friendly exploration of wastewater surveillance results.

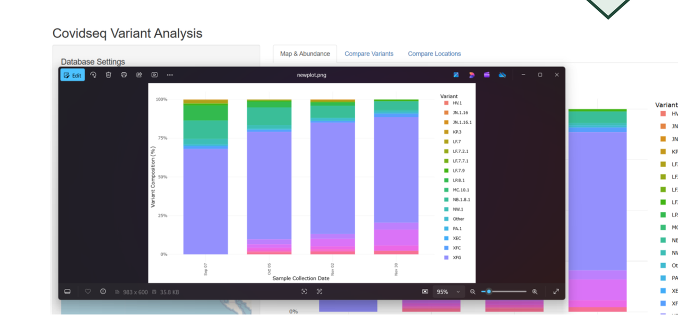


1. Secure browser access with no software installation.

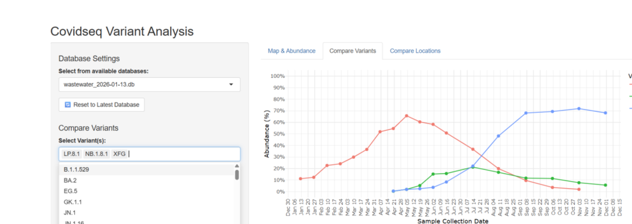


2. Geospatial sampling site selection with date-range filtering.

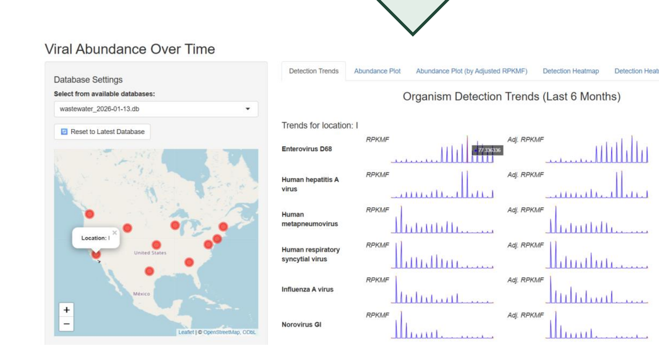
3. Interactive plots for pathogen and variant abundance trends.



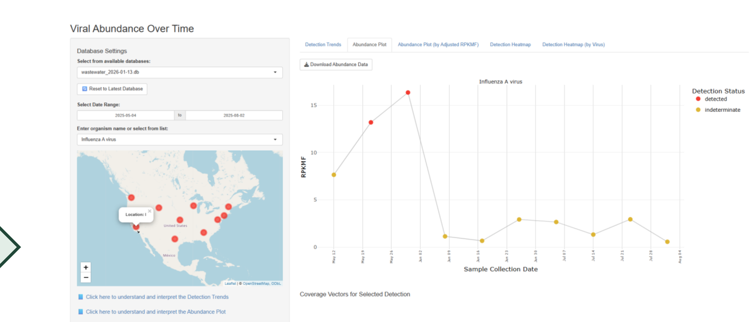
4. Quick export of plot snapshots for reporting.



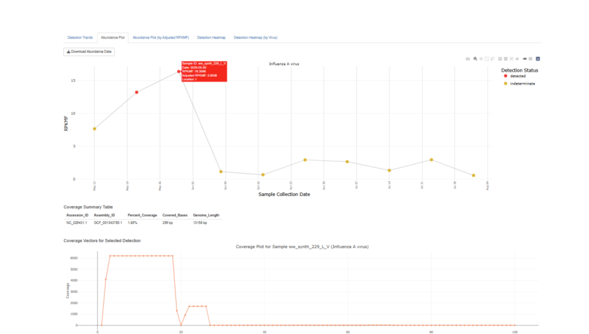
5. Visualize temporal variant abundance trends.



6. Immediate pathogen trend overview across sites.



7. Continuous monitoring of pathogen abundance over time.



8. Mouse-over details and click for genome coverage metrics

9. Heatmap summaries of pathogens detected across sampling sites.

CONCLUSIONS

- **Sustainable wastewater workflows** balance safety, flexibility, reproducibility, throughput, and data interpretability.
- One preserved sample and **co-purified DNA/RNA** extract can support multiple assay modalities.
- **WSB enables safer transport** and integrates with concentration workflows.
- Assay selection should be deliberate: **PCR-based assays** for speed and sensitivity, **hybridization capture** for added genomic context and **untargeted sequencing** for breadth and discovery.
- **Controls**, curated references and clear reporting are essential for actionable surveillance.

Citations
[1] Wastewater Stabilization Buffer: *https://www.zymoresearch.com/products/wastewater-sample-collection-bottle*
[2] Biomark X9 System, Standard Biotools: *https://standardbio.com/products-services/biomark-x9/*
[3] PEG-precipitation protocol: Bayati et al. (2022) *Identification and quantification of bioactive compounds suppressing SARS-CoV-2 signals in wastewater-based epidemiology surveillance. DOI:https://doi.org/10.1016/j.watres.2022.118824*
[4] Quick-DNA/RNA Viral Kits | MagBead: *https://www.zymoresearch.com/products/quick-dna-rna-viral-magbead*
[5] OneStep PCR Inhibitor Removal Kit: *https://www.zymoresearch.com/products/onestep-pcr-inhibitor-removal-new-kit*

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