POSTER NO. 2

Performance evaluation of dipstick-based wastewater surveillance of viruses through multi-operator Gage R&R study

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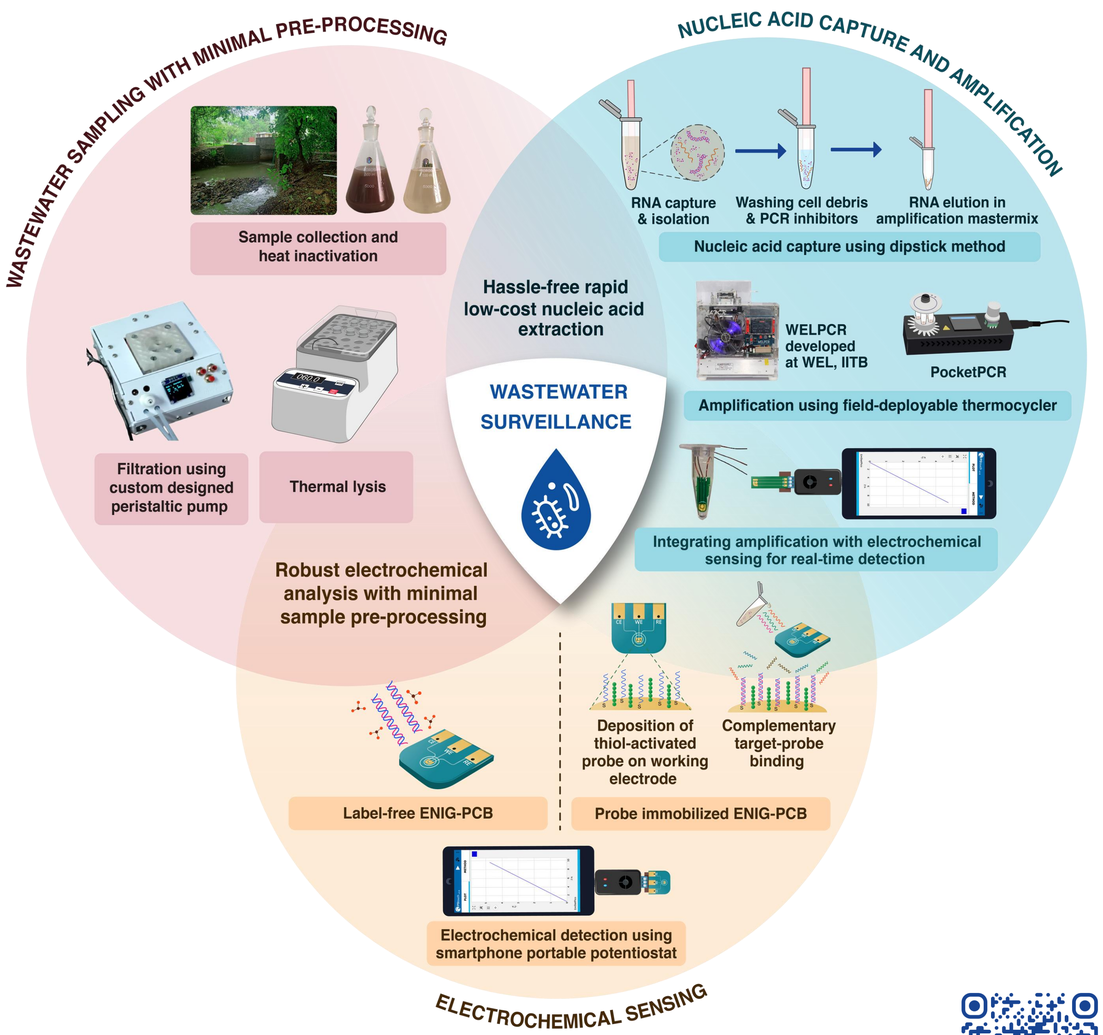
The COVID-19 pandemic highlighted the need of low-cost assays for automated wastewater surveillance, essential for early warning of frequent disease outbreaks and future pandemics. For large scale testing in densely populated low- or middle-income countries (LMICs) such assays need to be low-cost, rapid with high recovery efficiency. To address this constraint, we have devised an easy and rapid dipstick method for RNA isolation suitable for sub-millilitre sample volumes alleviating the complexities of existing RNA isolation methods.

Key contributions:

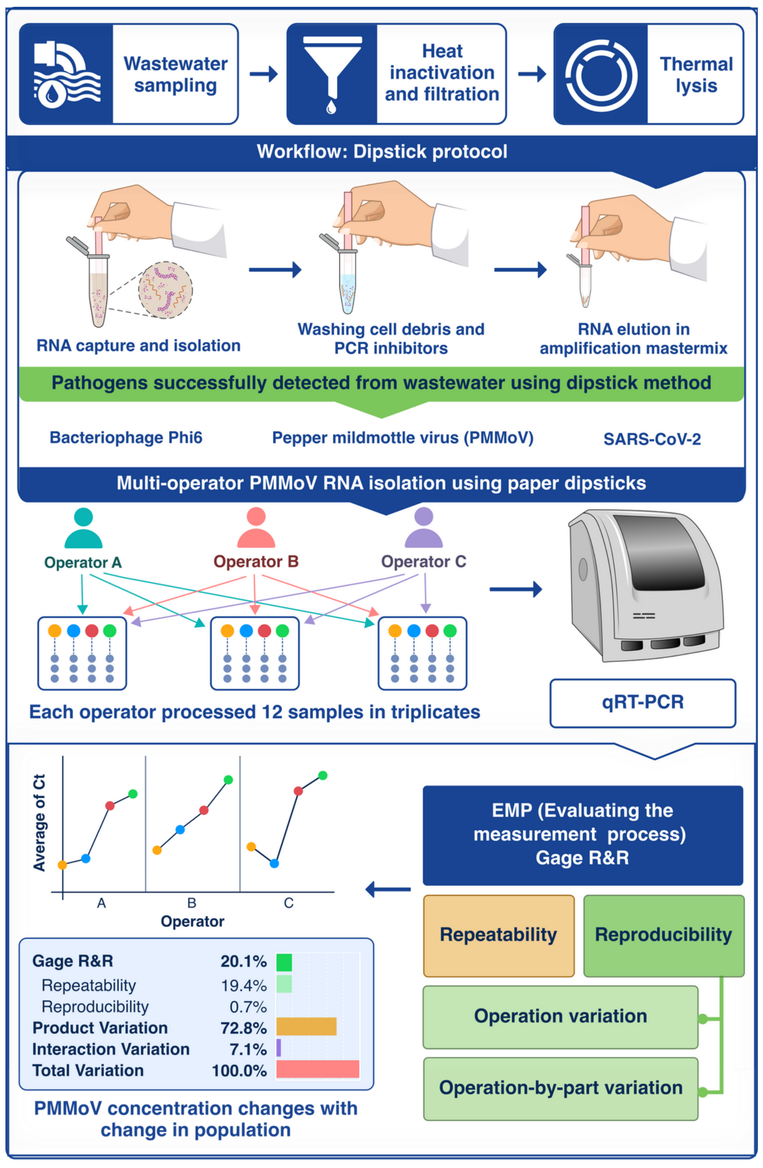
- **Validated efficiency:** Successful concentration and RNA isolation fom different pathogens such as SARS-CoV-2, PMMoV, and Phi6 from a wide range of wastewater samples with minimal sample pre-processing, comparable recovery efficiency to commercial kits.
- **Multi-operator Gage repeatability and reproducibility(Gage R&R) study:** Detected variations in PMMoV load associated with changes in population density with gage R&R <30%, classifying it as a second-class monitor according to EMP guidelines, indicating that the simplified dipstick method is within acceptable range of variability and reliable for field applications.
- **Automated dipstick preparation and assay operation steps that contributed to variability:** Simplified method does not impact Gage R&R, variability arises due to sample variation, confirming overall acceptability.

RESERACH OVERVIEW

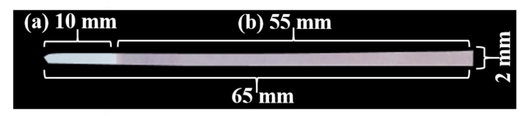


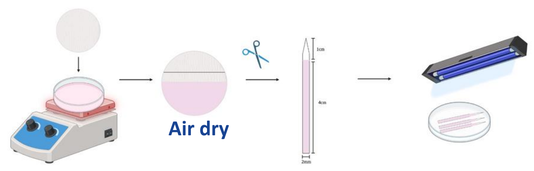
Scan to know more about our work on dipstick and electrochemical sensing

EXPERIMENTAL WORK

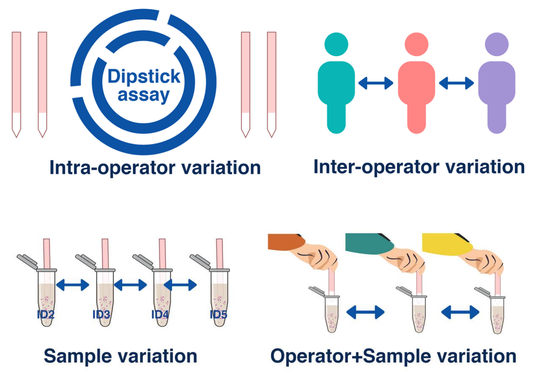


Dipstick preparation





Gage R&R study



Scan for a quick video guide for dipstick method and EMP gage R&R study

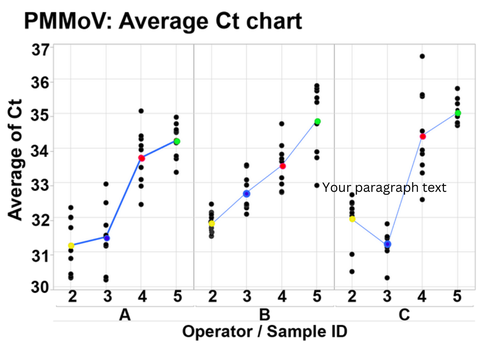
RESULTS

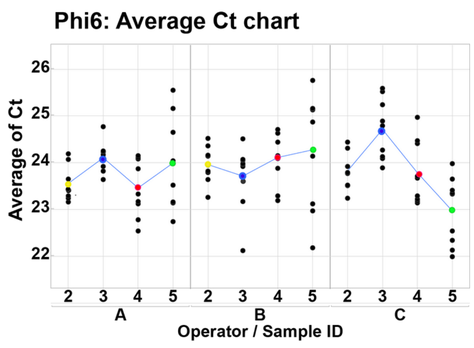
Pathogen detection from wastewater

METHOD	ORGANISM	Ct
Dipstick	SARS-CoV-2	33.74 ± 0.37
PowerWater Rneasy kit		34.74 ± 0.76
Dipstick	PMMoV	28.79 ± 0.24
PowerWater Rneasy kit		31.18 ± 0.37
Dipstick	Phi6	22.14 ± 0.36
PowerWater Rneasy kit		23.55 ± 0.21

- Standard curves were generated for each organism
- Process control (Phi6), and a negative pond water sample was used for SARS detection
- For multi-operator study 108 measurements 4 wastewater samples collected before and during semester break with each sample processed in triplicates, and tested by dipstick method thrice by 3 different operators

Multi-operator Gage R&R study





EMP Gage R&R Results

Component	% of Total
Gage R&R	20.1
Repeatability	19.4
Reproducibility	0.7
Product Variation	72.8
Interaction Variation	7.1
Total Variation	100.0

Variance component for PMMoV

Component	% of Total
Operator	0.7
Sample ID	72.8
Operator*Sample ID	7.1
Within	19.4
Total	100.0

EMP Gage R&R Results

Component	% of Total
Gage R&R	76.6
Repeatability	76.6
Reproducibility	0.0
Product Variation	0.0
Interaction Variation	23.4
Total Variation	100.0

Variance component for Phi6

Component	% of Total
Operator	0.0
Sample ID	0.0
Operator*Sample ID	23.4
Within	76.6
Total	100.0

CONCLUSION

Key challenges addressed:

- Low-cost, no capital cost, hassle-free nucleic acid extraction from wastewater.
- Reduced turn-over time compared to existing pathogen concentration and nucleic acid extraction methods such as PEG precipitation followed by kit based RNA isolation from 8+ hours to ~ 40 minutes.
- Minimal sample pre-processing.
- Comparable recovery efficiency with commercial kits.
- Field deployable with minimal operator-to-operator variability.
- Dipstick method can be integrated with an end point read out mechanism to deploy an on-field wastewater surveillance system

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