

LOW-COST, RAPID, INSTRUMENT-FREE DIPSTICK METHOD FOR WASTEWATER SURVEILLANCE IN LMICS

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AIMS LAB

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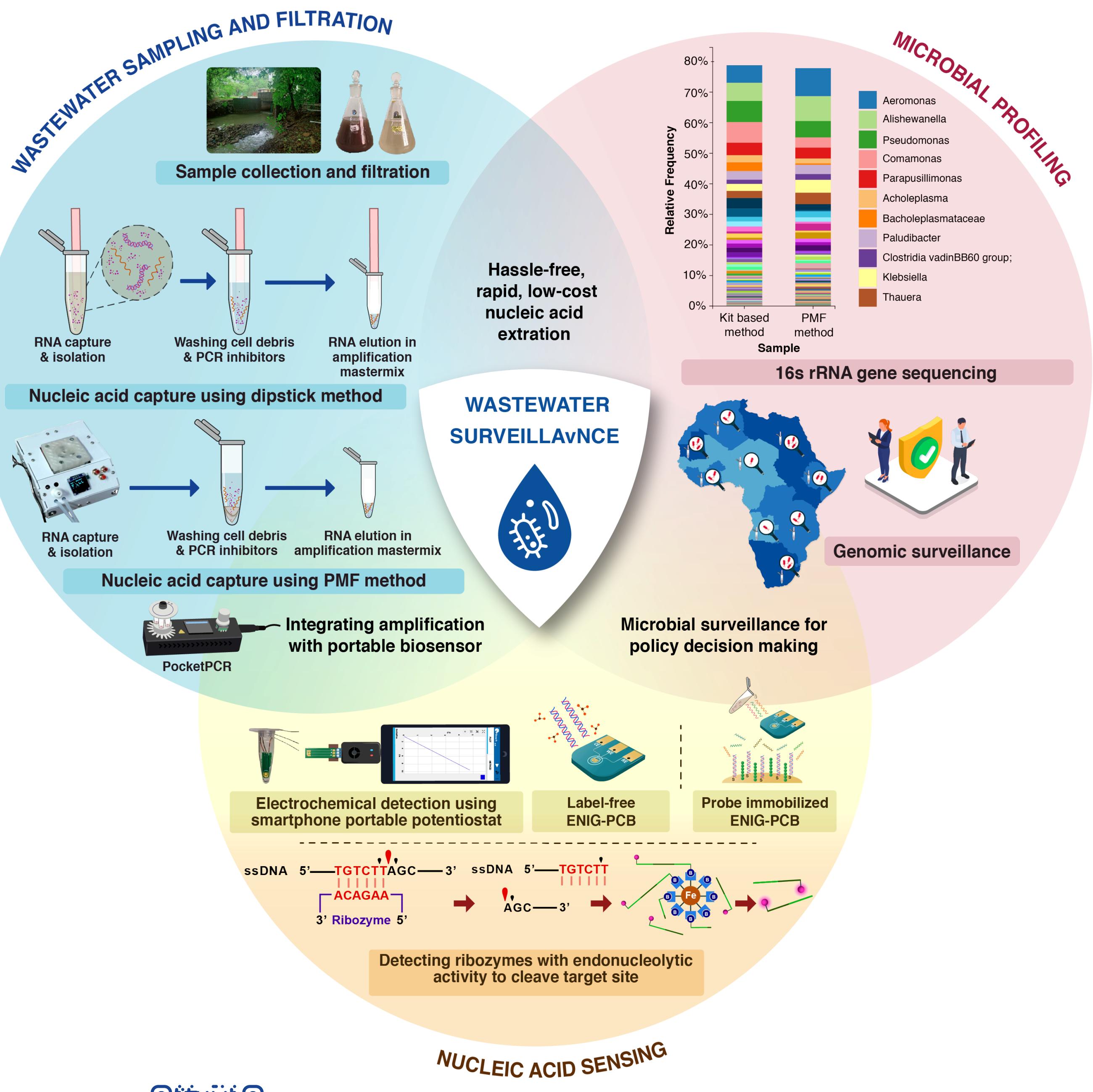
RATIONALE

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 low- or middle-income countries (LMICs) such assays need to be low-cost, rapid with high recovery efficiency.

KEY CONTRIBUTIONS

- Validated efficiency:** Successful nucleic acid isolation from different pathogens such as SARS-CoV-2, E.coli, 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%, indicating that the dipstick method is reliable for field applications.
- 16s rRNA gene capture:** Preliminary results demonstrate potential for sequencing-based detection, phylogenetic analysis, and microbial surveillance in wastewater.

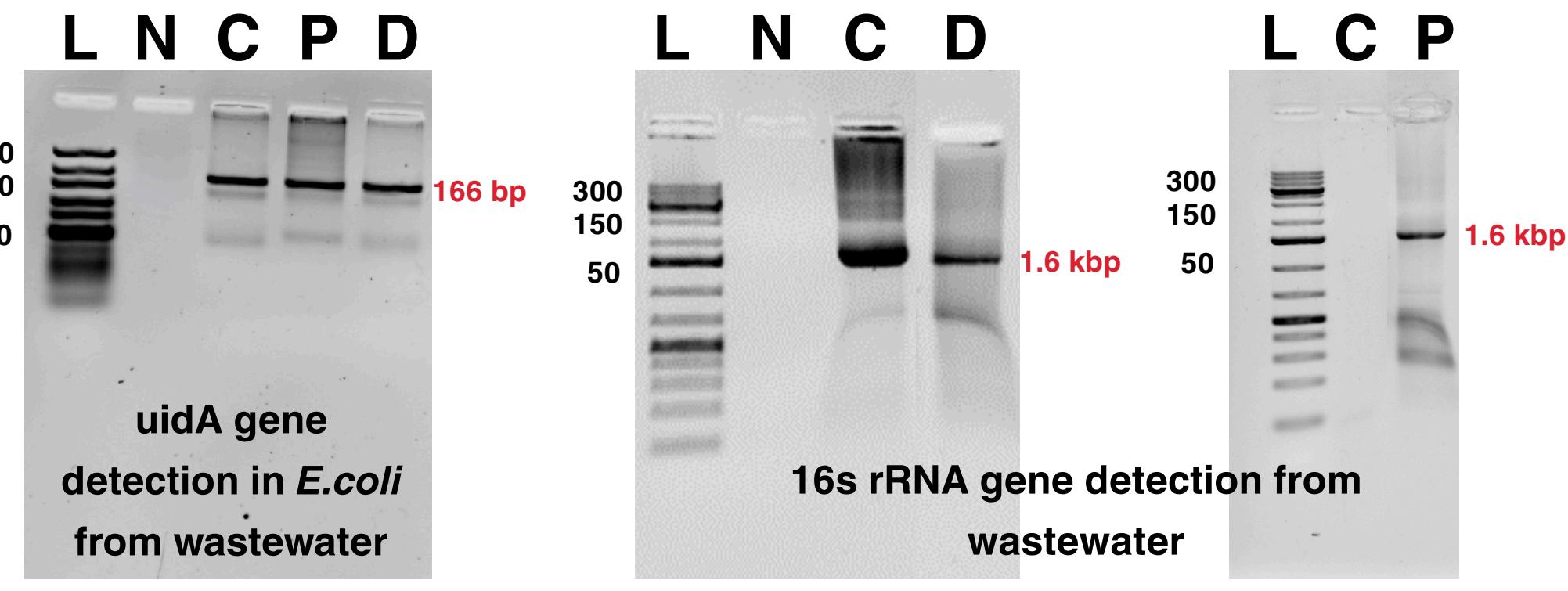
RESEARCH OVERVIEW



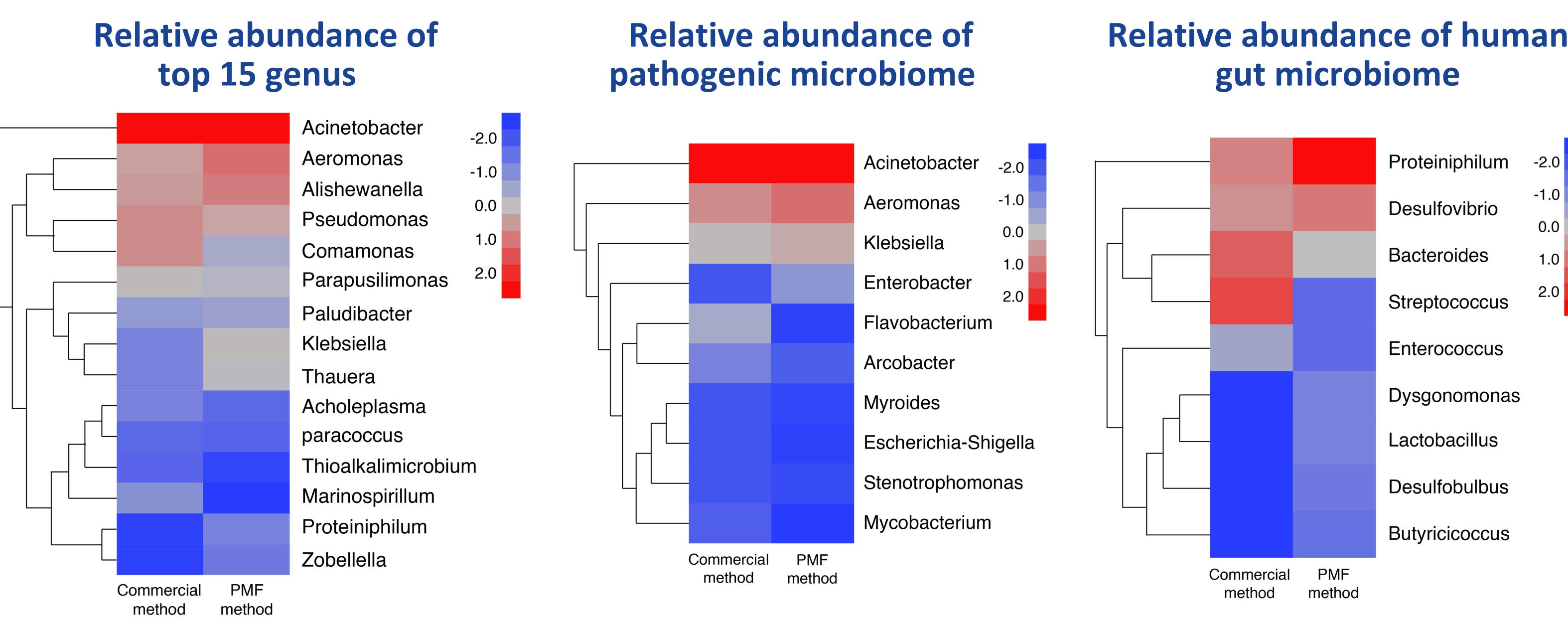
16s rRNA MICROBIAL PROFILING

- Dipstick method was validated against a commercial kit method for bacterial detection.

- 16s rRNA gene, ~1.6 kbp was successfully captured and amplified.



16s rRNA gene sequencing preliminary results with PMF method



- 16S rRNA gene sequencing targeting the V₃ – V₄ region was performed on wastewater samples collected from a treatment plant in Dombivli, Mumbai.
- Preliminary results using the commercial and PMF method identified 143 organisms across multiple phyla, including pathogenic, gut-associated, and environmental microbiota.

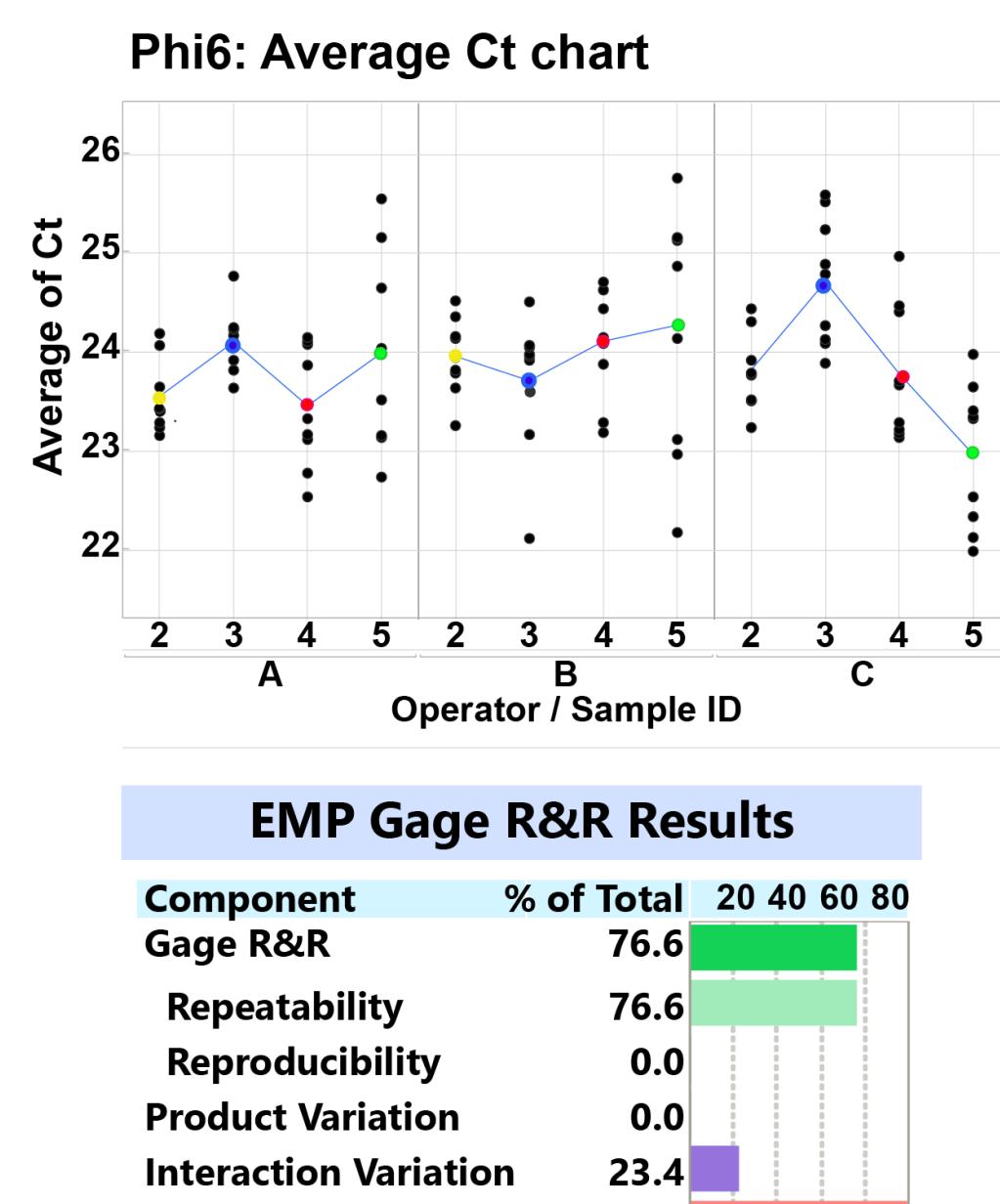
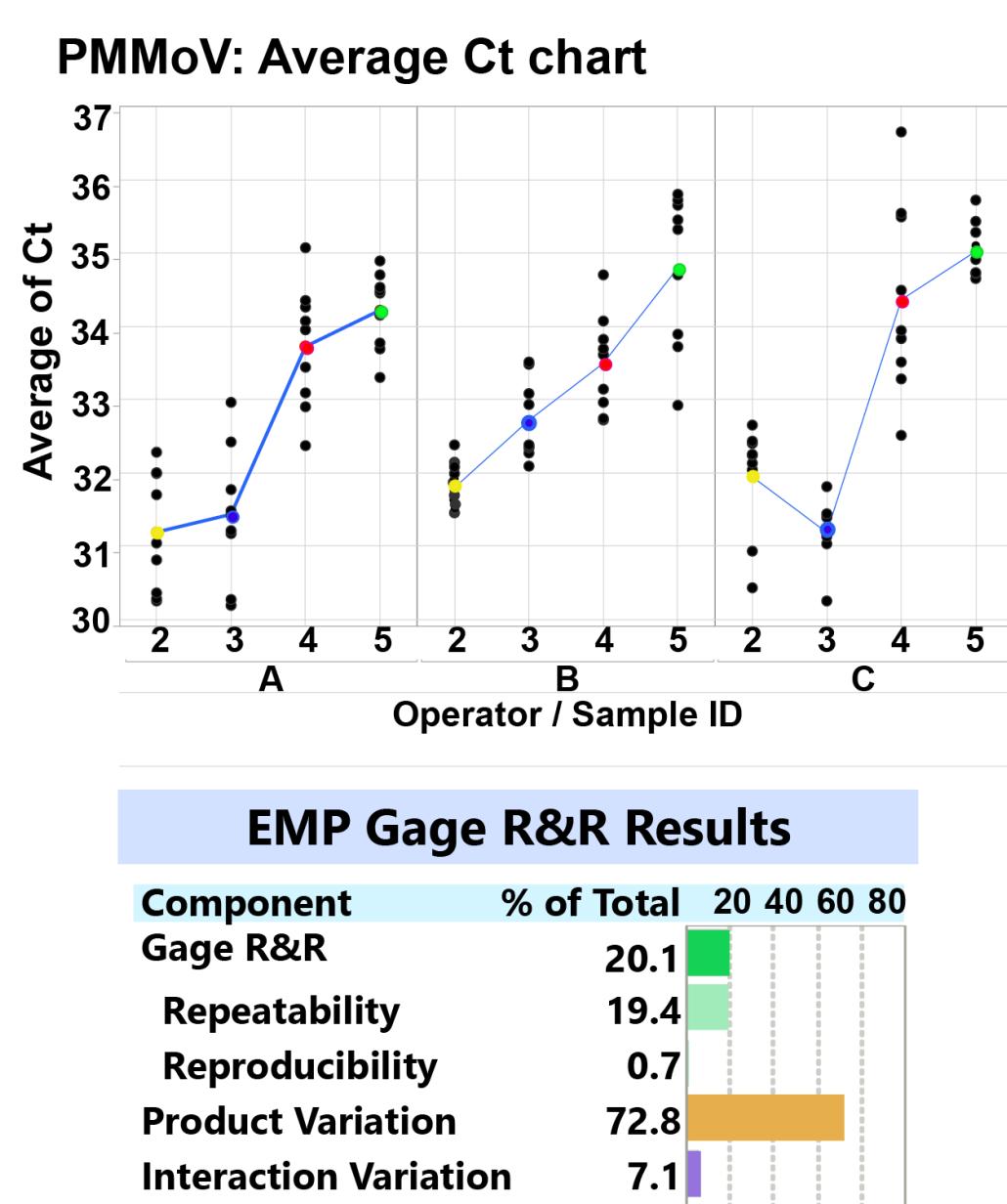
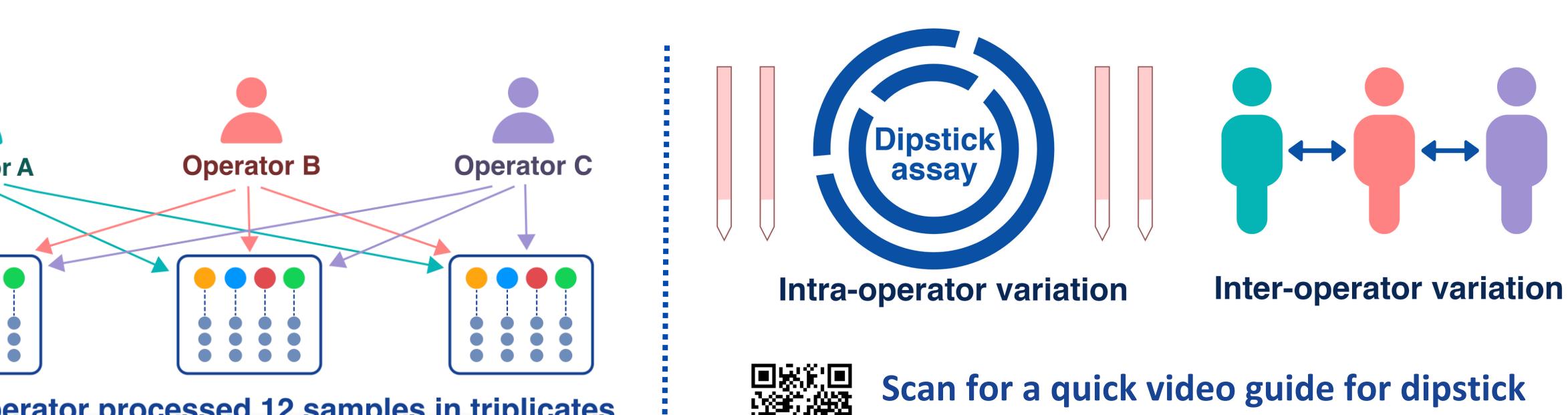
RAPID PATHOGEN DETECTION USING DIPSTICK METHOD

Benchmarking dipstick method

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



KEY CHALLENGES ADDRESSED

- Low-cost, no capital cost, hassle-free nucleic acid extraction from wastewater with minimal sample preprocessing.
- Reduced turn-around time from 8+ hours to approximately 30 minutes compared to existing pathogen capture and nucleic acid extraction methods.
- Comparable recovery efficiency with commercial kits.
- Field-deployable with minimal operator-to-operator variability.
- Potential use for 16srRNA sequencing and microbial surveillance.
- Dipstick method can be integrated with an end-point read-out mechanism to deploy an on-field wastewater surveillance system.

ACKNOWLEDGEMENTS