RAPIDSTICK: WASTEWATER SURVEILLANCE WITH HASSLE-FREE DIPSTICK RNA ISOLATION

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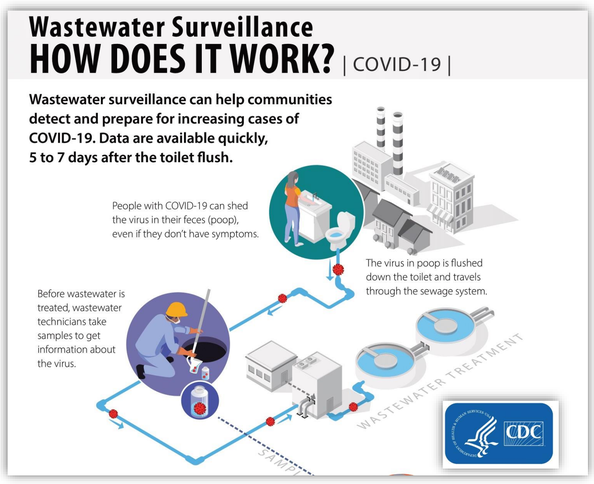
Wastewater surveillance has been used for decades to monitor pathogen spread at community level and the COVID-19 pandemic has reiterated its significance. The conventional process is tedious and involves pretreatment, lysis, RNA isolation and purification, amplification, and analysis. To address this constraint, we have devised an easy and rapid dipstick-based method for RNA isolation and purification, which alleviates the complexities of existing methods. The implementation of paper dipsticks for wastewater monitoring has not been explored till date. In this study, we investigate the viability of dipstick RNA isolation from wastewater by extracting RNA of Pepper Mild Mottle virus (PMMoV) and bacteriophage Phi6 (a popular SARS-CoV-2 surrogate) with minimal or no sample pre-processing from a sewage pumping station located at IIT Bombay, India. Additionally, with this method we have demonstrated isolation of spiked SARS-CoV-2 RNA from synthetic wastewater mimicking domestic sewage.

Motivation

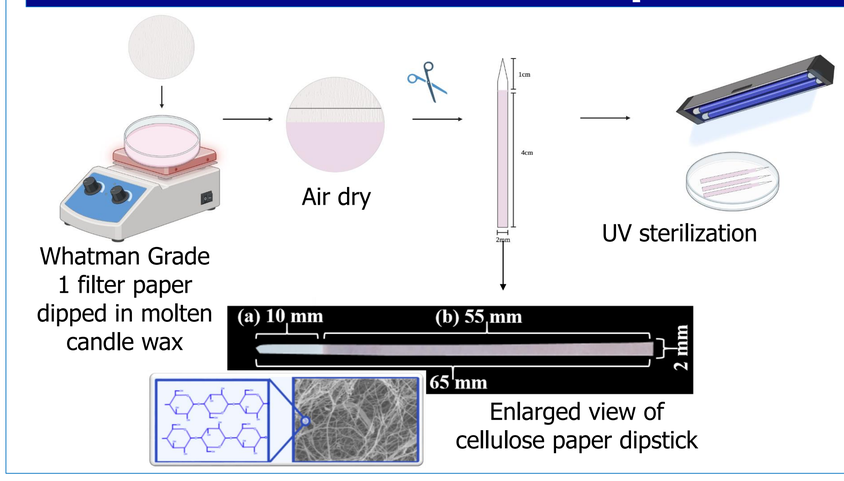


- Develop low-cost, field-deployable wastewater surveillance system

Research gap

Data from published literature related to COVID-19 (2019-21) based on Scopus web	
Research	57%
Review	43%
WBE	
Research	0.17%
60% from 5 countries: USA, UK, China, Italy, and India	

RAPIDSTICK: Preparation and Mechanism



- Selective capture due to retention kinetics
- Inhibitors are either not bound or released during washing
- RNA release occurs due to composition of amplification mix, liquid flow facilitated by bending and compressing of dipstick

Wastewater surveillance using RAPIDSTICK

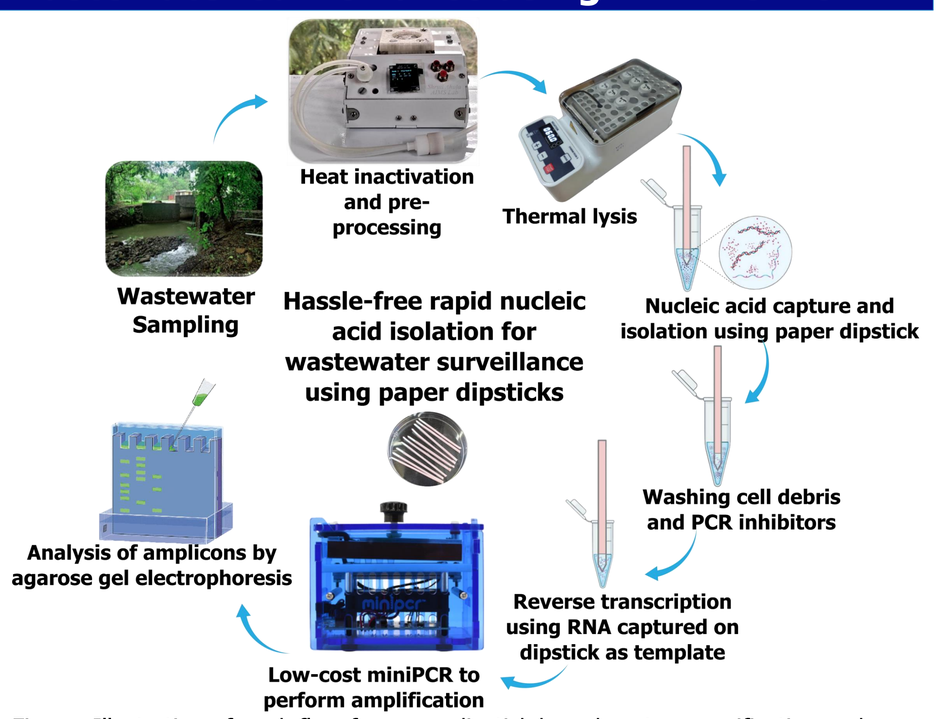


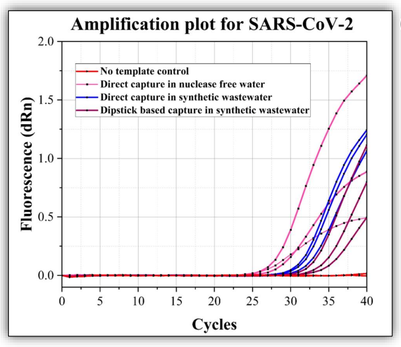
Figure: Illustration of work-flow for paper dipstick based capture, purification and amplification of nucleic acid

Isolation of SARS-CoV-2 RNA from synthetic sastewater

(a)

Sample	Ct value
Direct capture in nuclease free water	26.07 ± 0.63
Direct capture in synthetic wastewater	29.72 ± 0.49
Dipstick based capture in synthetic wastewater	32.04 ± 0.65

(b)



(c)

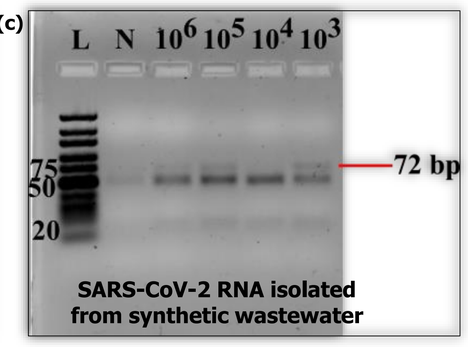


Figure: (a), (b) qRT-PCR Ct values and amplification plot for 10^6 copies/ml SARS-CoV-2 RNA isolated using direct capture and dipstick approach; (c) Agarose gel electrophoresis for 10^3-10^6 copies/ml SARS-CoV-2 RNA isolated from synthetic wastewater using paper dipstick (L: Ladder, N: No template control, 10^3-10^6: 10^3-10^6 copies/ml SARS-CoV-2 RNA)

- Synthetic wastewater mimics domestic sewage
- SARS-CoV-2 RNA spiked in synthetic wastewater at concentrations ranging from 10^3-10^6 copies/ml
- Paper dipsticks could detect ~ 10^3 copies/ml of SARS-CoV-2 RNA from synthetic wastewater

Virus RNA isolation and purification from wastewater using paper dipstick

Sample collection

Sample collection from Sewage pumping station, IIT Bombay, India

Sample	pH	Captured organism
Sample ID 1	8	PMMoV
Sample ID 2	7	Bacteriophage Phi6

PMMoV isolation from wastewater

- Samples pre-processed : 5 µm filtration and 1.6 µm filtration
- Both sample pre-processing resulted in successful detection of PMMoV

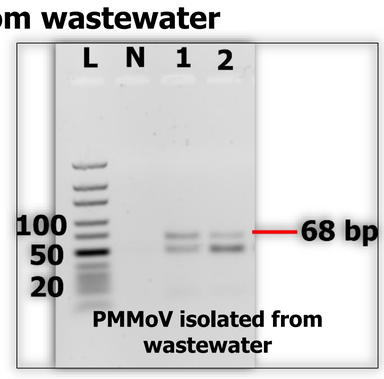
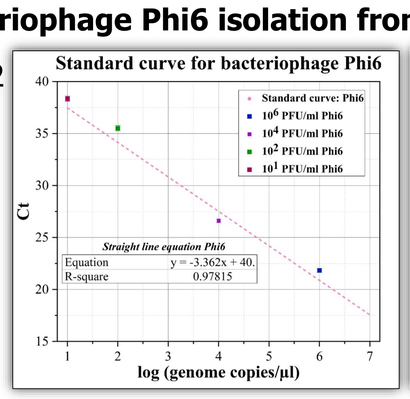


Figure: Agarose gel electrophoresis of PMMoV RNA isolated using dipstick; L: Ladder, N: No template control, 1: sample filtered through 5 µm filter, 2: sample filtered through 1.6 µm filter

Bacteriophage Phi6 isolation from wastewater

- Phi6: SARS-CoV-2 surrogate
- ~10^6 PFU/ml Phi6 spiked in wastewater successfully detected



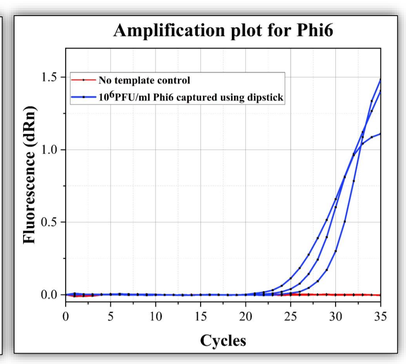


Figure: (a) Standard curve for Phi6; (b) Amplification plot for 10^6 PFU/ml Phi6RNA isolated from wastewater using dipstick

DNA detection

- ~10^4 CFU/ml *E.coli* spiked in artificial urine diluent detected using dipsticks
- Application: detect urinary tract infection

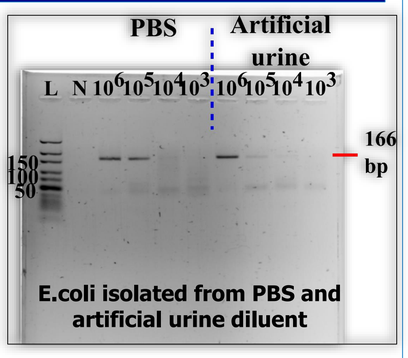
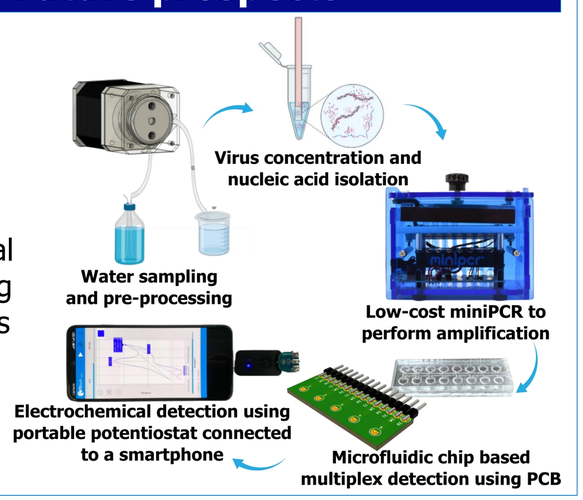


Figure: Agarose gel electrophoresis of E.coli DNA isolated from artificial urine diluent using dipsticks(L: Ladder, N: No template control, 10^3-10^6: 10^3-10^6 CFU/ml *E.coli* spiked in PBS and artificial urine diluent)

Future prospects

- ✓ Integrate dipstick with microfluidic platform for electrochemical detection using PCB electrodes



Key takeaways

- ✓ Dipsticks successfully purified nucleic acid from complex wastewater samples for the first time
- ✓ Dipsticks can be coupled with LFA for rapid microbial detection from wastewater with microbial load > 10^3 copies/mL

References

Mason, M.G., Botella, J.R. Rapid (30-second), equipment-free purification of nucleic acids using easy-to-make dipsticks. *Nat Protoc* **15**, 3663–3677 (2020).

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