

# On-field end-to-end wastewater surveillance for detection of SARS-CoV-2 using PCB electrodes

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## Wastewater Surveillance using PCB electrodes

Our group presents a low-cost PCB electrode based electrochemical sensor for detection of bacteriophage Phi-6 (popular surrogate for SARS-CoV-2) from lake water samples

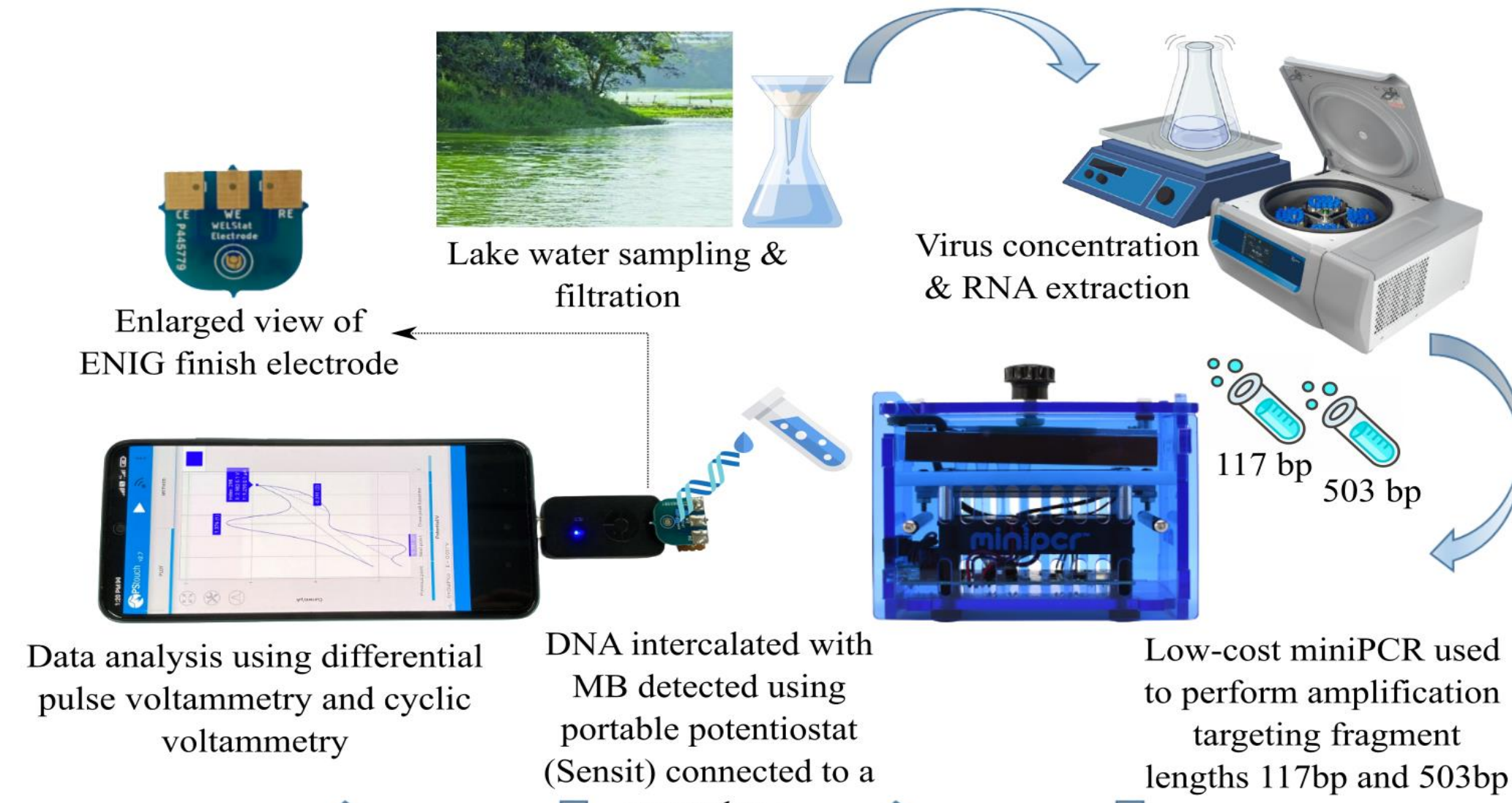


Figure: Illustration of work-flow for electrochemical detection of amplicons obtained from virus particles concentrated from lake water samples

## DNA fragments of dissimilar lengths and presence of salt exhibit different voltammetric response

- Increase in peak current for [DNA] < 1 ng/μl followed by a decreasing trend
- Presence of salt greatly reduces sensitivity for [DNA] < 1 ng/μl

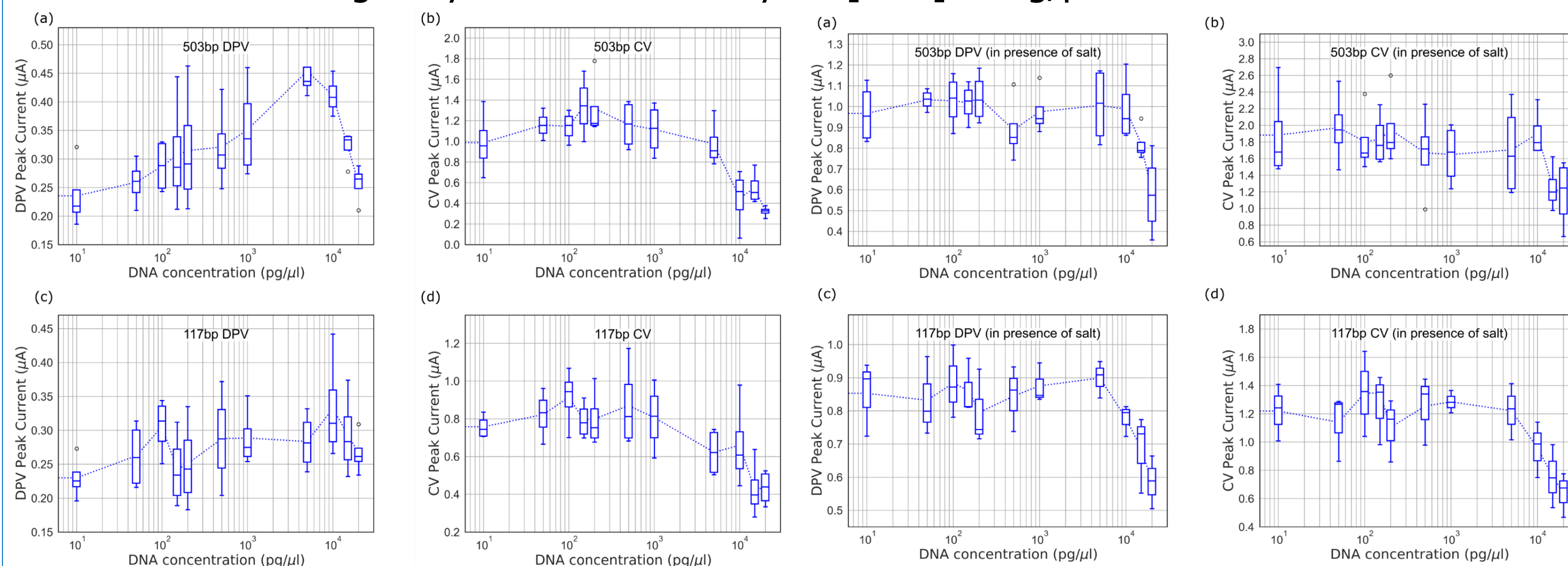


Figure: Peak current obtained from voltammogram for DNA complexed with 50 μM MB: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

Figure: Peak current obtained from voltammogram for DNA complexed with 50 μM MB + 2 mM MgCl<sub>2</sub>: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

## Validation using Optical absorbance

- Higher DNA concentration, gradual decrease in absorption signal
- Extent of reduction in absorption signal is comparatively less in presence of salt
- Reduction in absorption signal due to steric inhibition, n-n\* electron transition due to intercalation

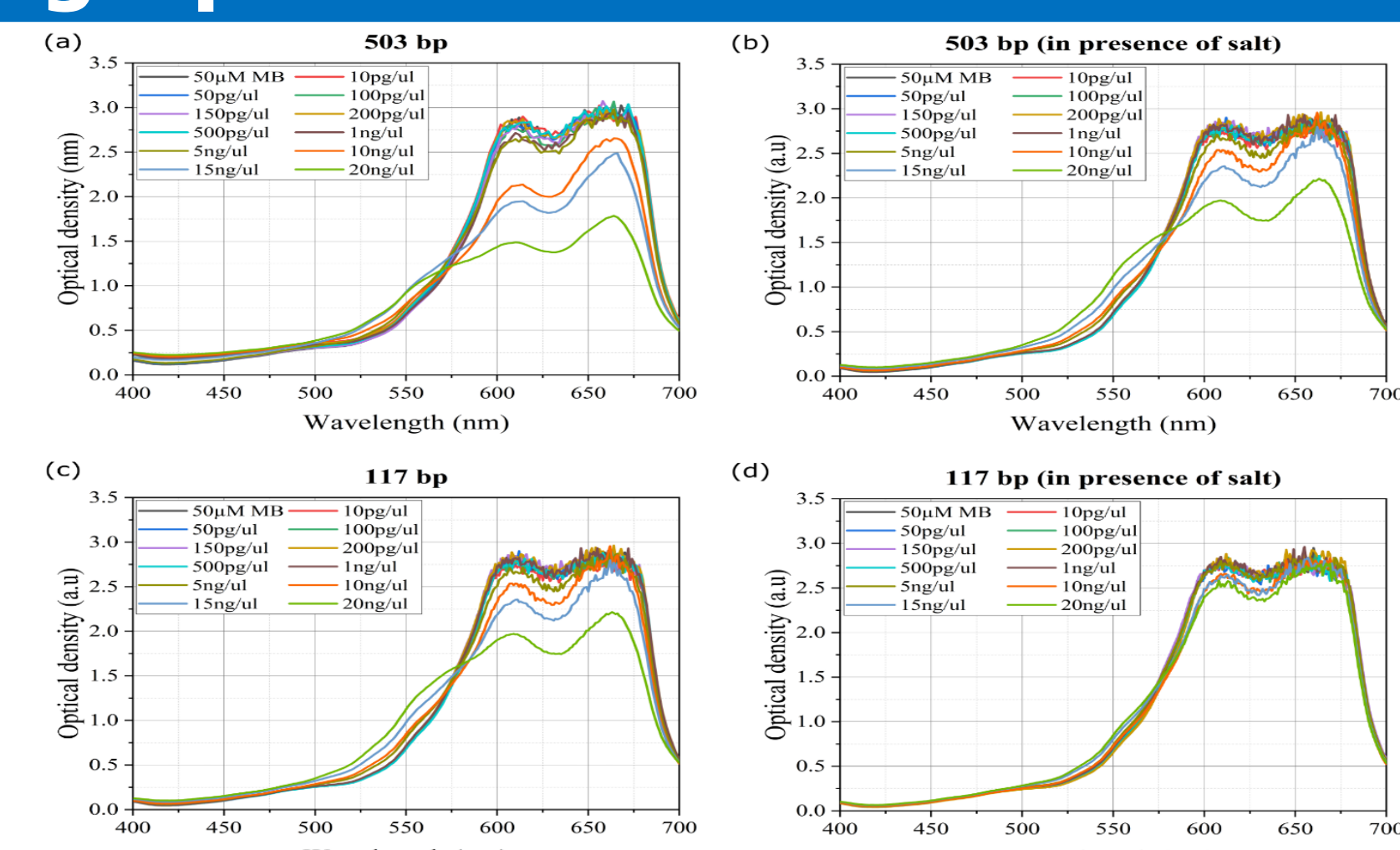


Figure: Absorption spectra (UV/vis Spectrometry) for 50 μM MB-DNA complex: (a) 503bp (b) 503bp + 2 mM MgCl<sub>2</sub> (c) 117bp (d) 117bp + 2 mM MgCl<sub>2</sub>

## Longer DNA fragment isolated from lake water detected with high sensitivity using unmodified PCB electrode

- Sensor utility was evaluated with Phi-6 spiked Powai lake water samples
- For longer amplicon NTC resulted in higher DPV and CV peak current relative to positive control (PC), and test samples

	Ct value for 117bp	Ct value for 503bp
NTC	31±0.04	30.75±0.44
Positive control	17.87±0.17	16.73±0.12
Test sample-1	20.65±0.11	19.41±0.08
Test sample-2	20.37±0.07	19.35±0.07
Test sample-3	21.1±0.28	20.51±0.2

Table: Ct values of samples as measured by qPCR

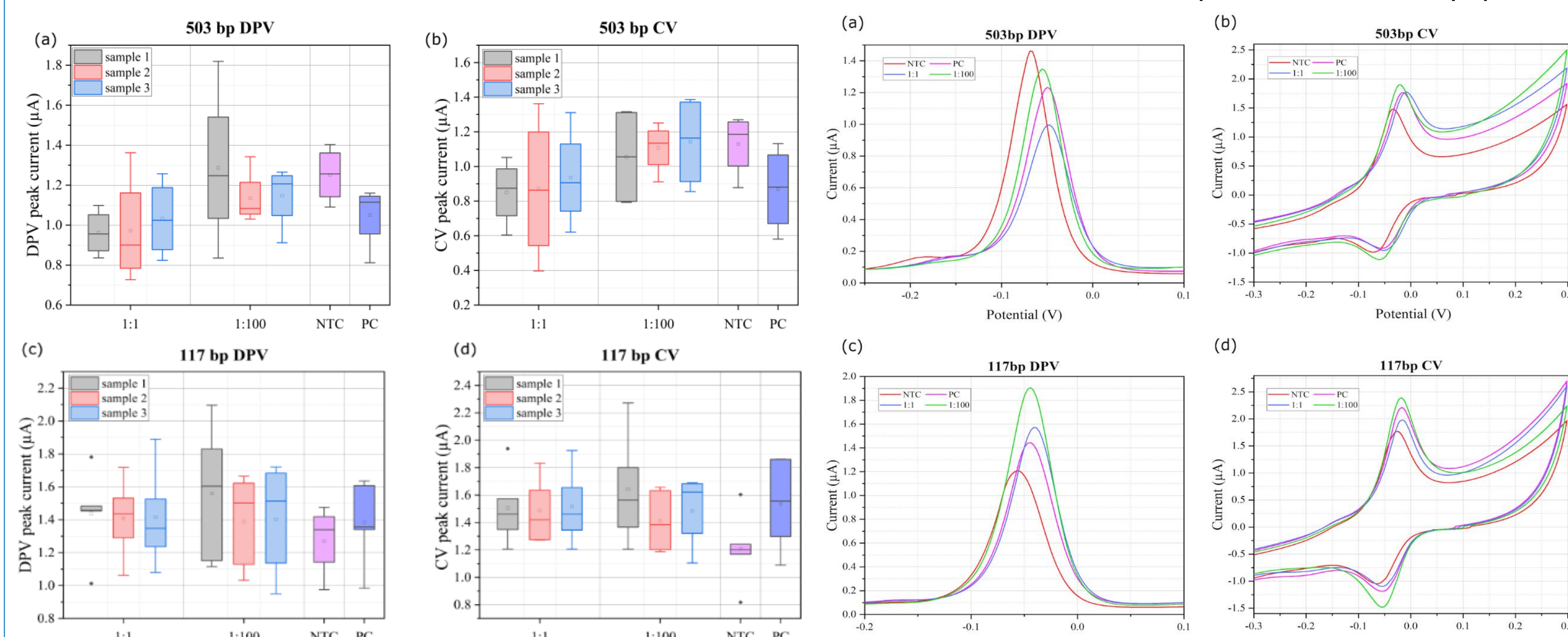


Figure: Peak current obtained from voltammogram for lake water samples complexed with 50 μM MB: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

Figure: Representative voltammogram for lake water samples complexed with 50 μM MB: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

## Key Takeaways

- Low-cost detection, potential for integration into fully-automated wastewater surveillance systems
- Minimal sample, no specific storage, no surface modification
- Manufacturing cost per ENIG PCB electrode: \$0.55 (INR 40)
- ENIG PCB based electrochemical sensing is suitable for longer fragments (≥ 500bp) at viral nucleic acid concentration isolated from lake water samples

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## Future Prospects

- ✓ Developing a water surveillance system to detect faecal indicators with minimal sample pre-processing
- ✓ Low-cost electrochemical detection
- ✓ Hassle free, rapid pathogen concentration and nucleic acid isolation
- ✓ Demonstrate high specificity and a limit of detection suitable for typical pathogen load in wastewater

Figure: Proposed work flow for integrated wastewater surveillance

## Preliminary Work

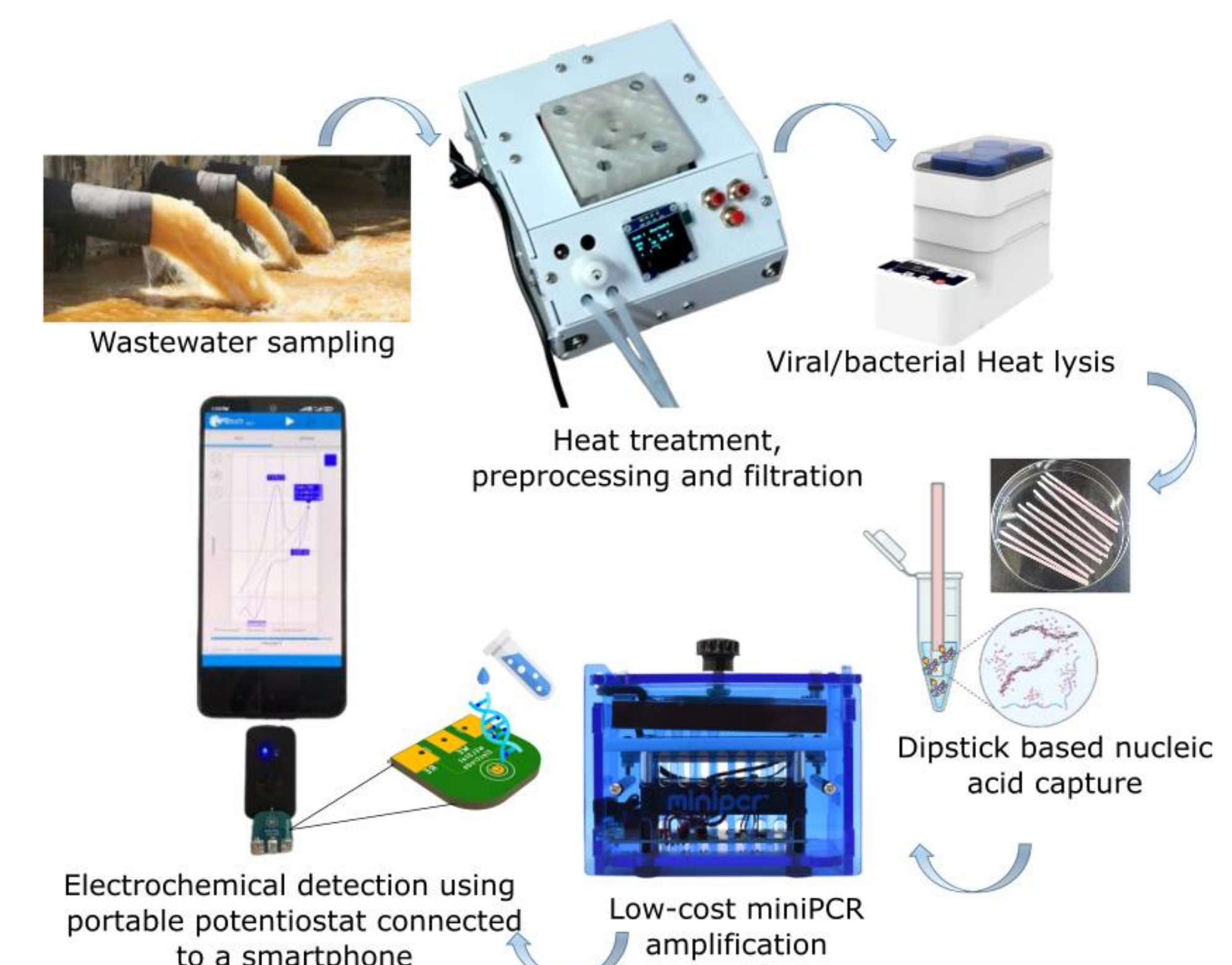


Figure: Preliminary testing workflow for nucleic acid isolation from wastewater using dipstick

## Preliminary Results

- ✓ Testing dipsticks with artificial urine samples spiked with *E. coli*
- ✓ Standard benchmarking with commercially available nucleic acid extraction kits for wastewater
- ✓ Testing the specificity and sensitivity of the dipstick assay in artificial urine and wastewater

\*\*\* This assay can also be used to detect urinary tract infections

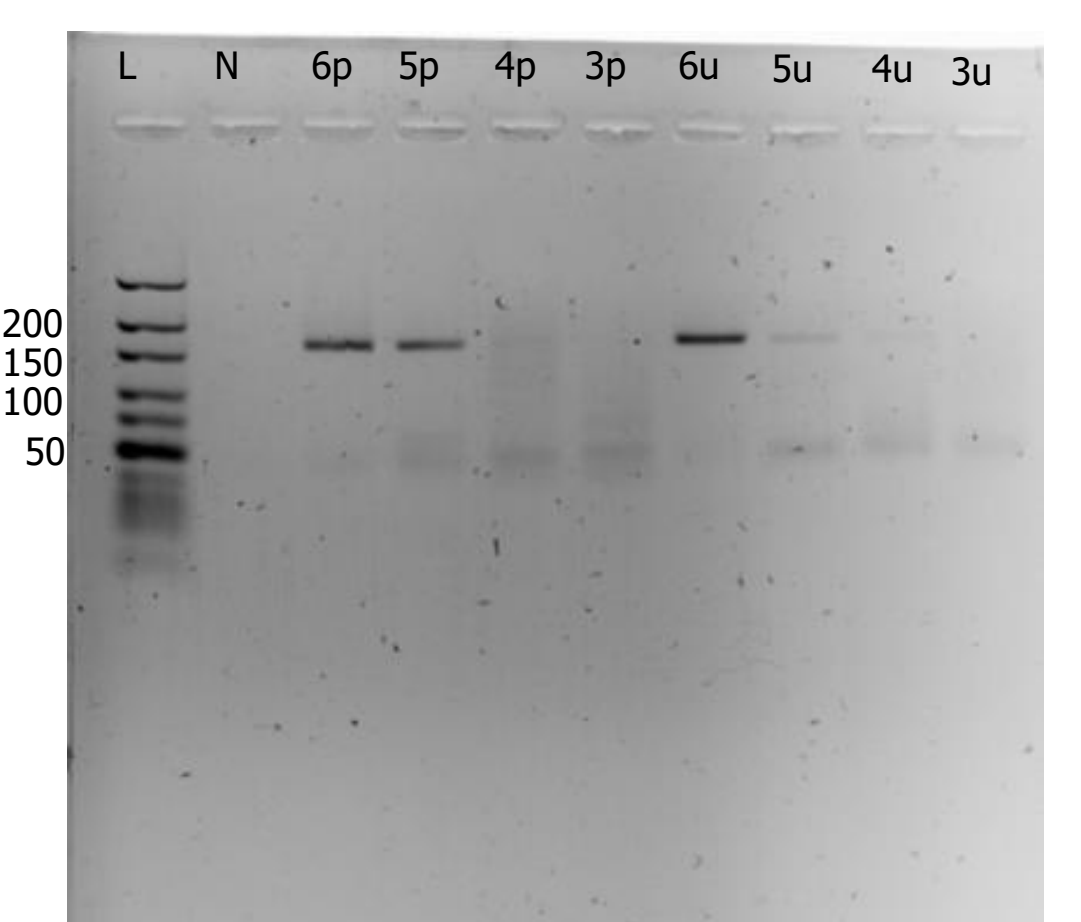


Figure: Agarose gel electrophoresis for E coli spiked PBS and urine  
L: Ladder; N: NTC; 6p to 3p: PBS samples spiked from 10<sup>6</sup> to 10<sup>3</sup> cfu/ml E coli; 6u to 3u: Artificial urine samples spiked from 10<sup>6</sup> to 10<sup>3</sup> cfu/ml E coli

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