

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

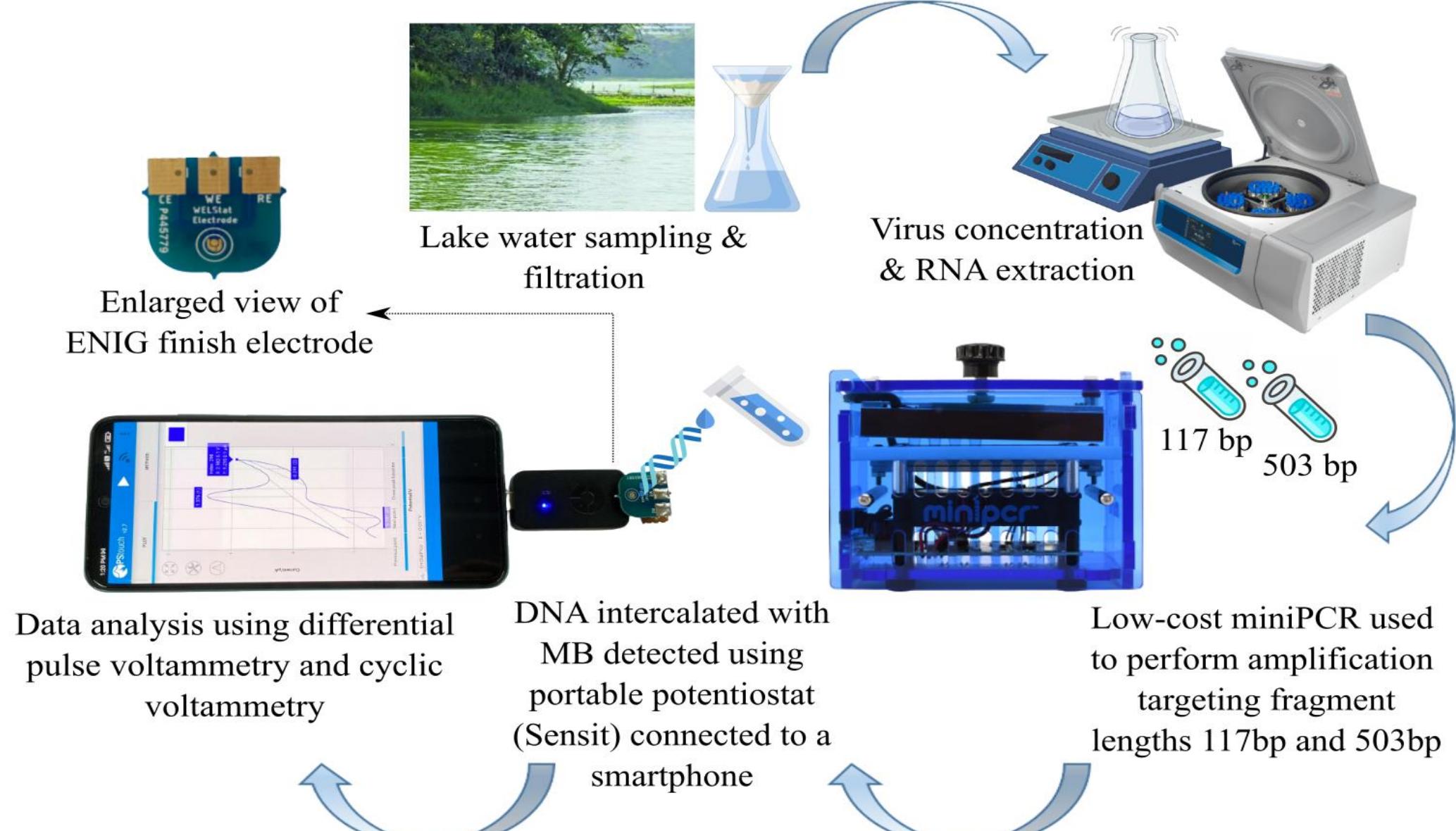


Figure: Illustration of work-flow for electrochemical detection of amplicons obtained from virus particles concentrated from lake water samples

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

- Increase in peak current for $[DNA] < 1\text{ng}/\mu\text{l}$ followed by a decreasing trend
- Presence of salt greatly reduces sensitivity for $[DNA] < 1\text{ng}/\mu\text{l}$

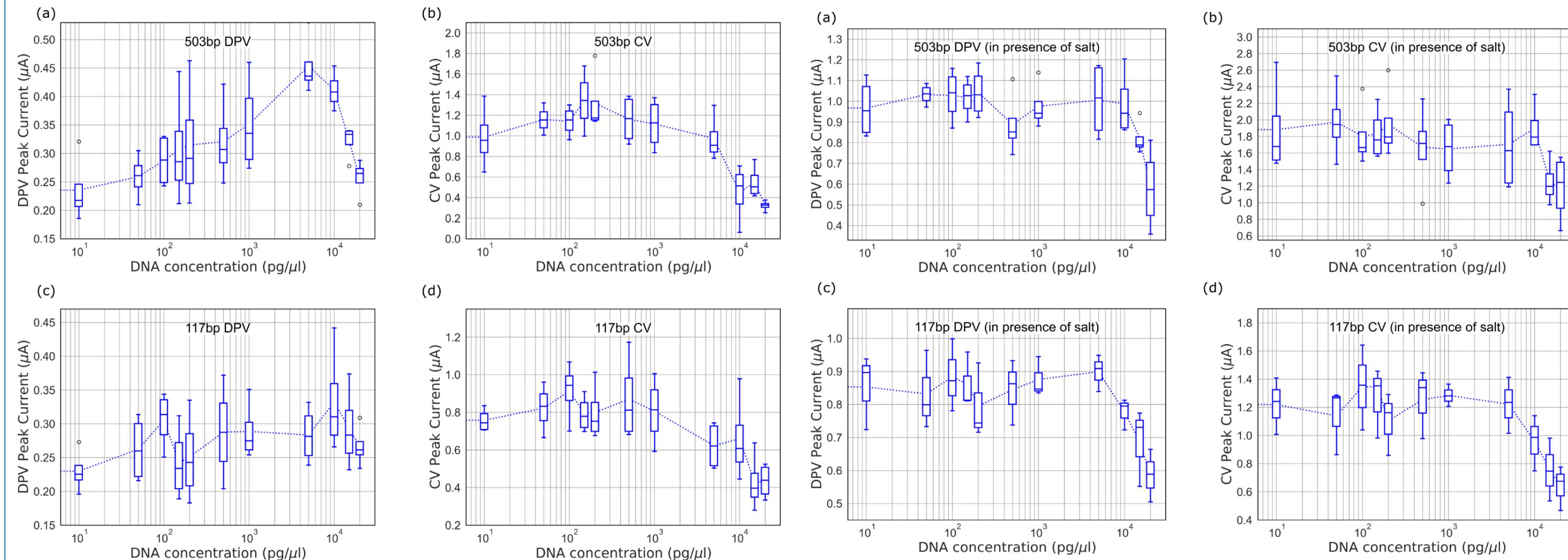


Figure: Peak current obtained from voltammogram for DNA complexed with 50μM MB: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

Figure : Peak current obtained from voltammogram for DNA complexed with 50μM MB+ 2mM MgCl₂: (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

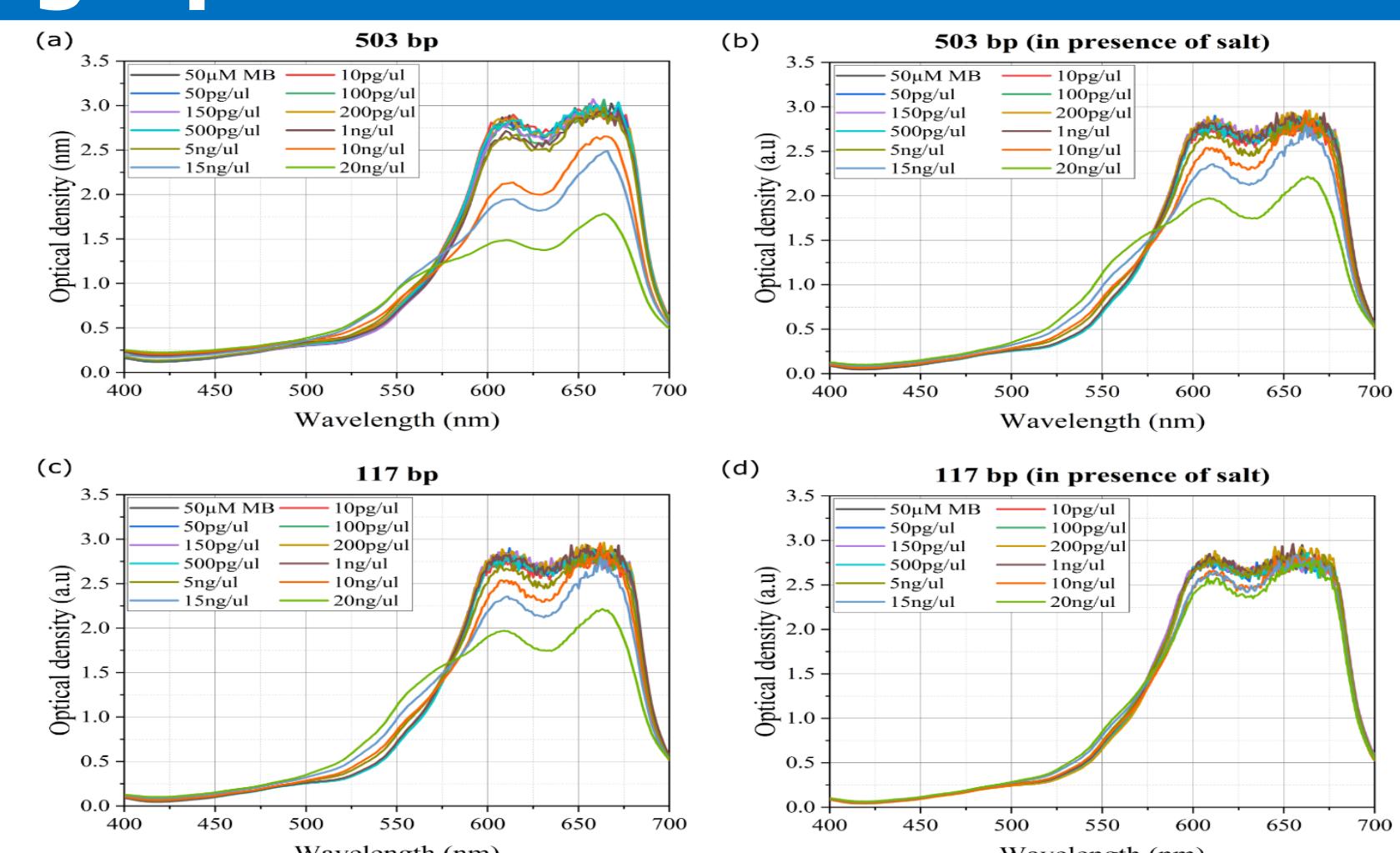


Figure : Absorption spectra(UV/vis Spectrometry) for 50μM MB-DNA complex: (a) 503bp (b)503bp+2mm MgCl₂ (c)117bp (d) 117bp+2mm MgCl₂

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

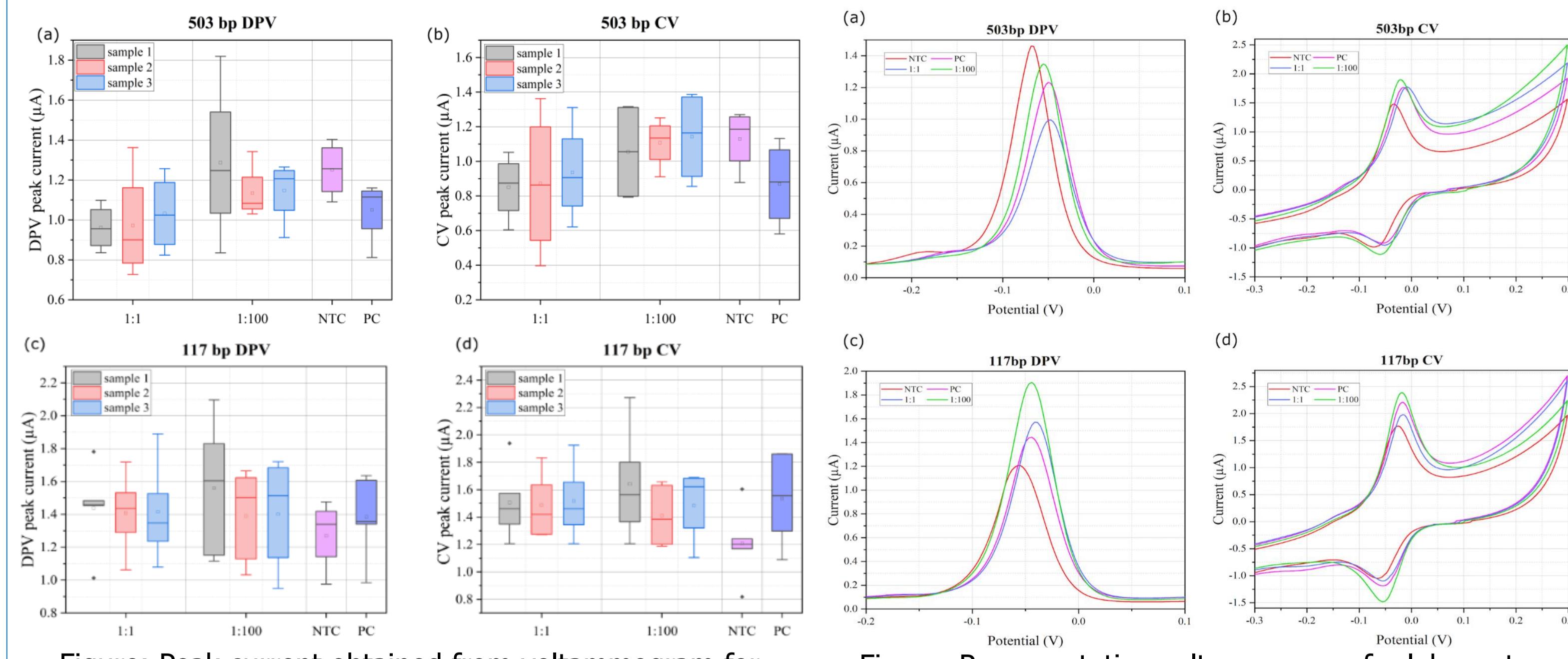


Figure: Peak current obtained from voltammogram for lake water samples complexed with 50μM MB: (a) 503bp DPV, (b) 503bp CV, (c) 117bp DPV, (d) 117bp CV

Figure: Representative voltammogram for lake water samples complexed with 50μM 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 ($\geq 500\text{bp}$) 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

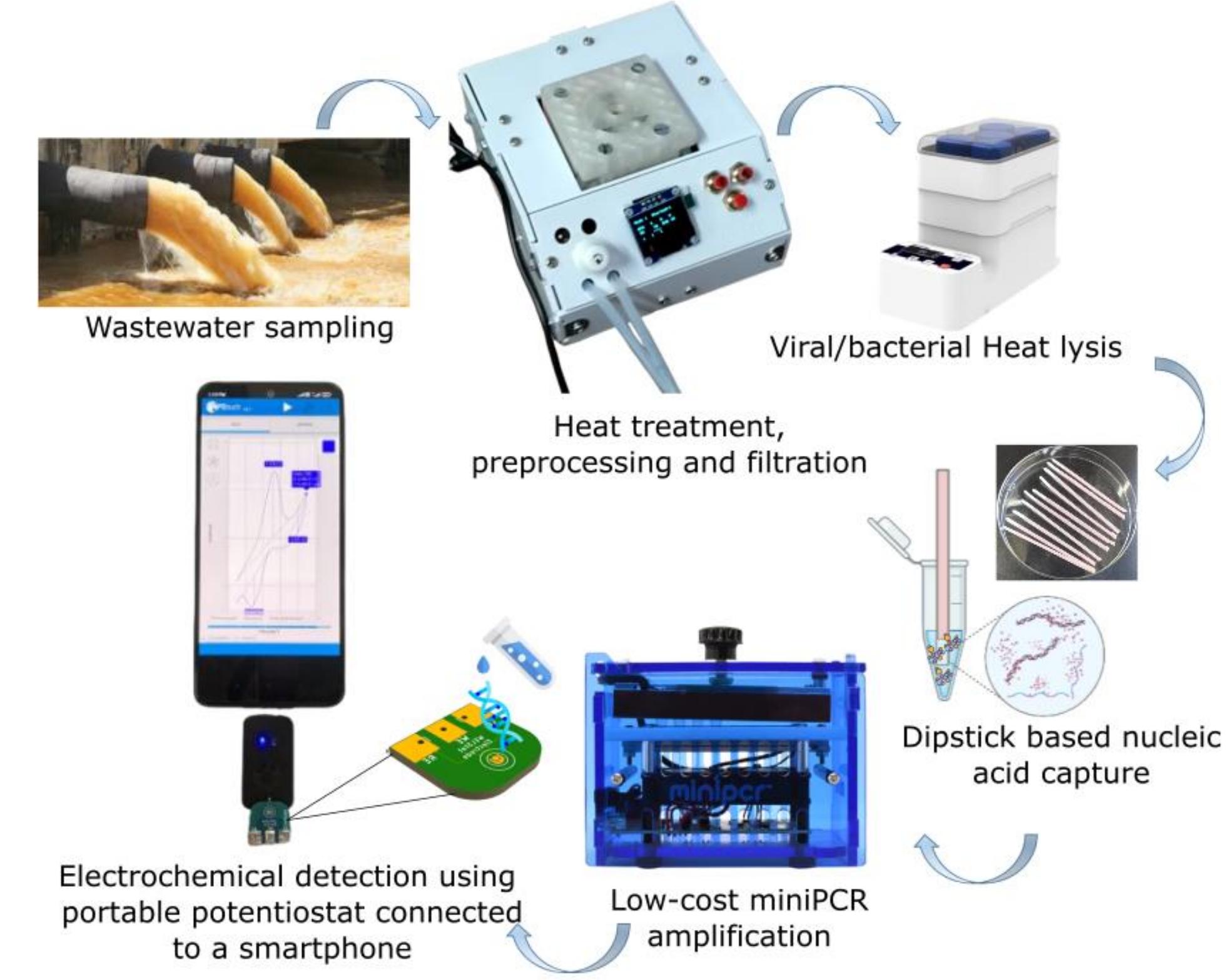


Figure: Preliminary testing workflow for nucleic acid isolation from wastewater using dipstick

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

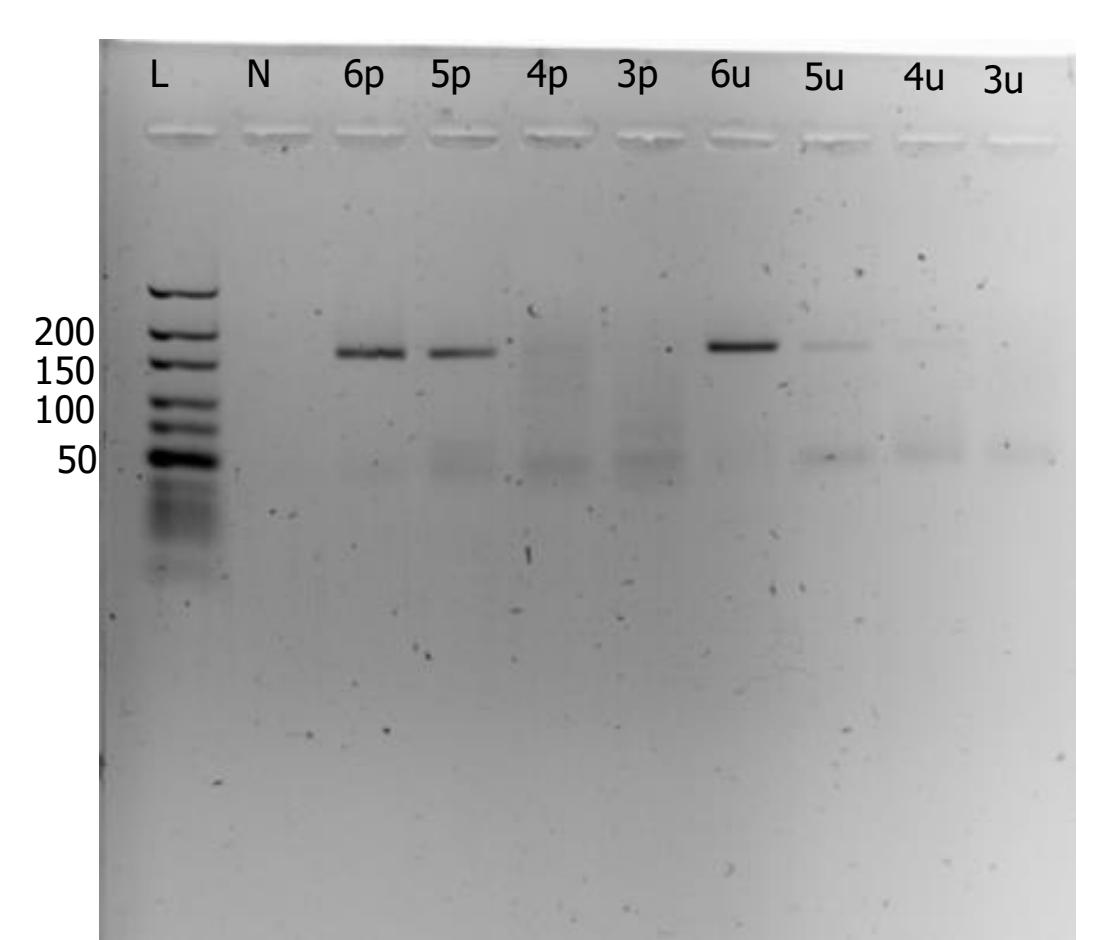


Figure: Agarose gel electrophoresis for E coli spiked PBS and urine
L: Ladder; N: NTC; 6p to 3p: PBS samples spiked from 10^6 to 10^3 cfu/ml E coli; 6u to 3u: Artificial urine samples spiked from 10^6 to 10^3 cfu/ml E coli

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