Miniaturized and Rapid Electrochemical Immunosensor for the detection of Tuberculosis Based on carbon screen-printed electrodes

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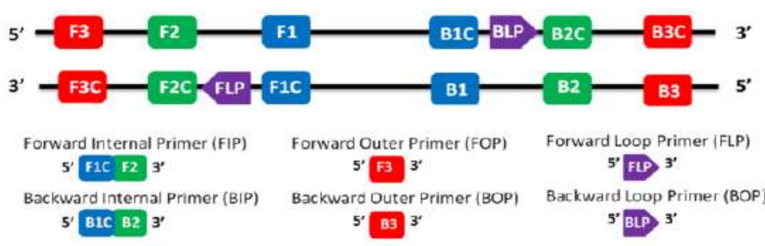
Introduction

Tuberculosis (TB) is the first infectious disease reason of mortality, it continues to a global burden with around 2.5 million new patients and .3 million deaths annually. The world is facing an unprecedented pandemic. A quarter of the world's population is infected and, between 2020 and 2021, it is predicted that 10 million people will have fallen ill, 3 million will not have been diagnosed or received care, and more than 1 million— mainly the most vulnerable—will have died.1 This pandemic is not COVID-19 but tuberculosis, TB remains one of the world's top infectious killers [1]. Analyzing, testing, and early diagnosis can contain TB-causative bacterium, Mycobacterium tuberculosis (Mtb). Biosensing is the deck that allows rapid, sensitive, and selective detection [3] which in turn can serve the purpose for rapid and precise detection of TB. In our work, based on miniaturized sensing strategies, focuses on detecting the TB by using affordable cost-high efficiency processes with the help of loop-mediated isothermal amplification (LAMP) [2] analysis and screen printed electrodes (SPE) implemented on a commercial potentiostat. The device measures the current response generated from the interaction between the target molecules and the SPE using cyclic voltammetry (CV). The system (LAMP-EC) proposes a promising electrochemical sensor for the detection of TB that can be clinically adopted by dint to its feasibility and high sensitivity. Our purpose is to make it an integrated lab-on-chip quantitative rapid point-of-care in both high- and low-resource settings across the TB endemic regions.

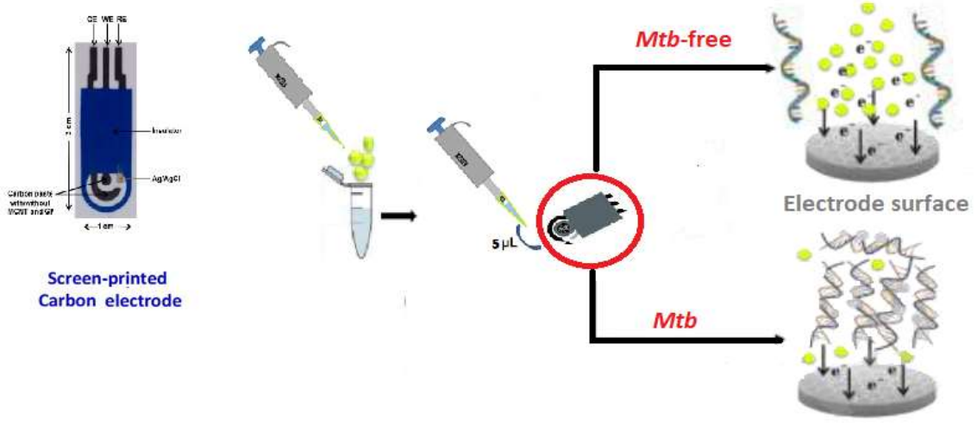
Methods

In this work, a system will be implemented to analyze LAMP reaction by an electrochemical device using carbon screen-printed electrodes (SPE) and commercial potentiostat for cyclic voltammetry analysis in real time electrical response which make from it a rapid electrical response for the detection of Mtb. To detect LAMP amplicons using the electrochemical method, the oxidation signals were measured using cyclic voltammetry (CV) in the potential range between -0.15 and 0.57 V with a step potential and a scan rate of 100 mV/s, respectively. Electrochemical experiments were carried out using the potentiostat. The data were analyzed using PSTrace 5.8 software package.



Location and sequence of primers.

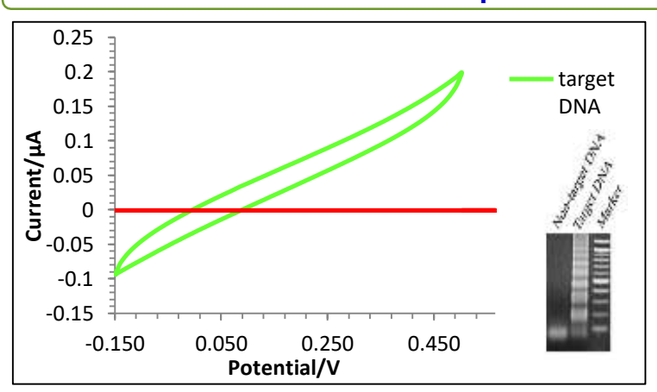
Electrode Functionalisation Protocol



Experimental Setup

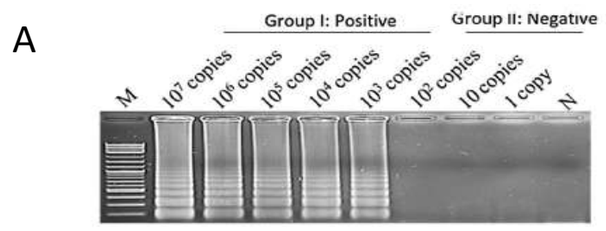
Results

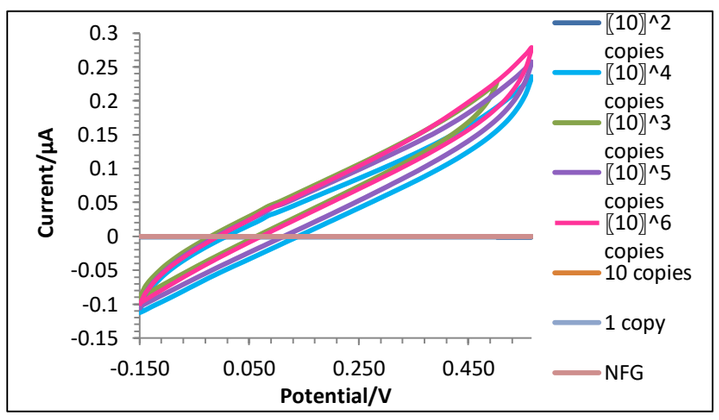
Mtb-Free and Mtb reaction response



CV showing the responses generated from the Mtb LAMP reaction in the presence of target Mtb-DNA(green line) or a lack thereof (red line) (as measured by the mini-potentiostat. The CV was recorded at a scan rate of 100 mV/s, showing the current response(i) with respect to the applied potential (E). Inset: AGE results corresponding to the tested samples: non-target DNA (H2O) and target DNA (Mtb).

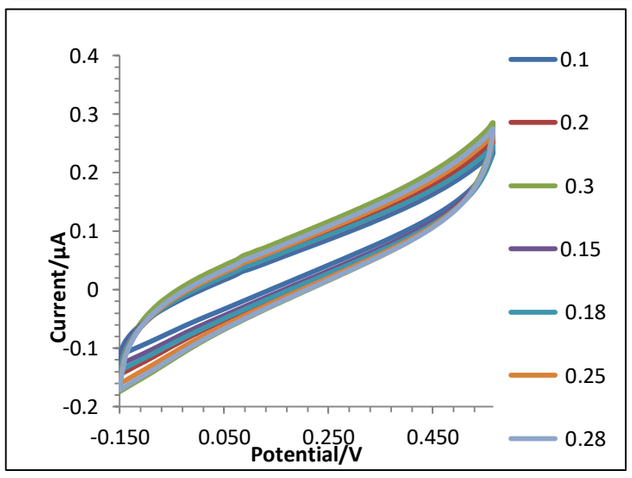
Different concentration of LAMP reaction response

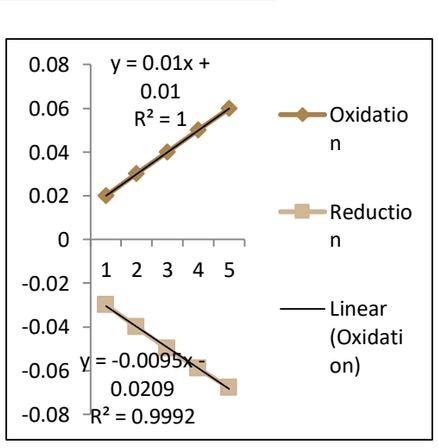




A) AGE results of LAMP products amplified from various amounts of Mtb-plasmid templates. Lanes M and N: molecular marker and DNase-free water (negative control), respectively. The LAMP products were grouped based on the presence (Group I) or absence (Group II) of DNA amplicons. (B) CV of the samples listed in (A)..

Reproducibility Reaction of SPE whit LAMP reaction response





Conclusions

This work seeks to further improve an isothermal amplification technique by method integration of LAMP whit DNA readout technology that allows novel diagnostics to be implemented in low-resource settings. It was observed that the system (LAMP-EC) offers a rapid end-point quantitative analysis of specific DNA amplicons. This in turn changes the oxidization derived current values of the Mtb-LAMP reaction making it a suitable electrochemical sensor for the detection of Tuberculosis.

REFERENCES

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ACKNOWLEDGMENTS

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