Simple and Low Cost Antibiotic Susceptibility Testing for *Mycobacterium tuberculosis* using Screen-Printed Electrodes

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Abstract

One quarter of the global population is thought to be latently infected by *Mycobacterium tuberculosis* (TB) with it estimated that 1 in 10 of those people will go on to develop active disease. Due to the fact that *Mycobacterium tuberculosis* (TB) is a disease most often associated with low and middle income countries it is critical that low cost and easy to use technological solutions are developed which can have a direct impact on diagnosis and prescribing practice for TB. One area where intervention could be particularly useful is antibiotic susceptibility testing (AST). In this work presents a low cost, simple to use AST sensor which can detect drug susceptibility on the basis of changing RNA abundance for the typically slow growing *Mycobacterium tuberculosis* (TB) pathogen in 96 hours using screen-printed electrodes and standard molecular biology laboratory reactionware. In order to find out sensitivity of applied sensor platform, different concentration (10^8-10^3 CFU/mL) of *M. tuberculosis* was performed and Limit of detection (LOD) and limit of quantitation (LOQ) was calculated as $10^{3.82}$ and $10^{11.59}$ CFU/mL, respectively. The results display that it was possible to detect TB sequences and distinguish antibiotic-treated cells from untreated cells with a label-free molecular detection. These findings pave the way for development of a comprehensive, low cost and simple to use AST system for prescribing in TB and mdrTB.

Keywords: Antimicrobial resistance profiling, antibiotic susceptibility testing, electrochemical sensing, Mycobacterium tuberculosis (TB).

1. Introduction

The United Nations (UN) and World Health Organization (WHO) have been continuously stating that Antimicrobial resistance (AMR) is a major global issue [1]. As an example, AMR detected in *Mycobacterium tuberculosis* (TB) samples is still high and continues one of the most urgent global health challenges worldwide. Since Bacillus Calmette–Guérin (BCG) vaccination has limited protection [2], early detection and proper treatment of TB patients is very important to minimize the rate of transmission and emergence of drug resistance. The WHO reports that the overall detection of tuberculosis cases is still less than 60% in low-income countries and only 66% worldwide [3]. Every year, about 3 million people with active TB are not diagnosed or notified under the national TB control program [4, 5]. For this reason, drug susceptibility testing (DST) is significant to guide right treatment, but less than 1 in 20 new TB patients have access to DSTs [6]. Therefore, there is an urgent need for inexpensive, practical and efficient diagnostic tools that can be used at the Point of Care (POC).

TB is member of mycobacterium genus, which contains pathogens known to cause serious illnesses in mammals, such as leprosy and tuberculosis. TB does not take up the Gram Stain because there is a waxy coat surrounding the cell wall [7, 8]. It is a well-known and set up bacterium that through the years has evolved some of techniques and resistance mechanisms which permit it to propagate between entire human populations [9]. It is expected that TB latently infects less than a quarter of the world's population (around 1.7 billion individuals) [10] and a crucial aspect of its behaviour is its slow metabolism which means it can take a long time to culture and analyse patient samples and draw definitive conclusions about drug resistance and susceptibility profile.

Antimicrobial susceptibility tests (ASTs) are mainly examined by traditional methods like disk diffusion, dilution (agar and broth) and E-test. In the literature, Coban et al. reported the susceptibility of the bacteria to Isoniazid, rifampicin, ethambutol, and streptomycin antibiotics using Blood Agar Validation for TB [11]. Hall et al., presented an antimicrobial susceptibility test of resistant TB using microtiter assay [12]. These methods are quite safe, however; the main limitations are low sensitivity, complex sample preparations, lack of automation, and growth dependence [13]. Nowadays, studies have shifted towards molecular detection strategies such as Matrix-Assisted Laser Desorption Ionization-Time of Flight

Mass Spectrometry (MALDI-TOF MS), Polymerase chain reaction (PCR), and electrochemical methods.

Current methods of AST are categorized into phenotypic and genotypic methods. Phenotypic methods require the isolation of pathogens from patients and their subsequent culture in the presence of antibiotics which takes long time like diffusion, dilution [14]. Though often used in clinics and research laboratories, these tests also require larger quantities of samples with relatively low sensitivity. In contrast, genotypic methods are often faster and more effective, but they require a knowledge of the mechanisms and genes that lead to certain resistances [15]. PCR and DNA microarray-based susceptibility diagnostics are central aspects of genotype-based AST methods which detected the existence of genes or gene products that induce resistance against certain antibiotics [14, 15]. These techniques, however, are often expensive and time-consuming. In addition, there are several emerging sensor approaches, including fluorescent, colorimetric, electrophysiology [16], microfluidic [17], and electrochemical sensors. Single molecule approaches like electrophysiology provides high specificity however it is not transferable to clinic. Electrochemical sensors can accomplish AST in short time due to its high specificity for bacterial pathogens in blood culture. 16s rRNA can be detected in real-time and without prior purification [18].

Current AST methods require culture-based by approximately 10 days for the case of TB [19]. In addition, molecular methods are expensive and cumbersome to be used, especially in resource-constrained settings. Biosensor technology is an alternative approach with short analysis time, low cost, easy operation, technical simplicity, and real-time data collection [20]. An approach primarily based totally on a biosensor that detects resistance profiles could be an ideal solution [20-22]. A novel biosensor to come across drug resistance in TB strains within 2-3 days could be a totally vital device so one can assure the fast choice of right anti-TB drugs for powerful remedy and to eliminate transmission of the ailment and resistance development.

Electrochemical biosensors are ideal method for molecular diagnostics with label-free detection [23, 24]. A number of electrochemical antibiotic susceptibility tests have been demonstrated recently, with the topic becoming quite popular, including gel-modified screen-printed AST systems[25], lab-on-a-chip based systems involving microfabricated devices [26] and specially modified sensors

surfaces, sensitive to bacterial growth through crystalline layers including resazurin [27]. To identify the molecular pathogens and efficient monitoring, we have previously demonstrated electrochemical biosensors detection with specific hybridization of 16S rRNA oligonucleotide sequences of bacteria [28, 29]. Herein, this work presents an electrochemical detection methodology to determine antibiotic susceptibility of slow-growing TB by measuring the changes in antibiotic incubation-dependent nucleic acid levels. To do so, we have applied a low cost commercially available electrode platform, screen-printed gold electrode (SPGE) and evaluated the AST after bacterial incubation with and without Isoniazid. The AST assay for TB was evaluated with Isoniazid, one of the most established treatments for TB with isoniazid. The mechanism of action of Isoniazid is based on inhibition of bacterial growth through disruption of mycolic acid synthesis within 24 hours [9]. The proposed methodology is quick, cost-effective, can easily be performed in different settings and crucially involves standard off the shelf microbiology, molecular biology and electrochemical reagents and components and so will translate well into real clinical work, especially in resource limited settings where specialised device manufacture and reagent storage is not available.

2. Materials and Methods

2.1. Target Preparation

Targets were prepared through a process of culture, incubation, lysis, and fragmentation. Middlebrook 7H9 broth (Sigma - Aldrich, USA) was used as the medium for culture of the TB strain (ATCC 25177/H37Ra). 2% glycerol (Sigma-Aldrich, USA) and 0.5% Tween (Sigma - Aldrich, USA) were added in the medium to prevent bacterial coagulation. Then TB at a density of 0.5 McFarland (as an initial concentration) was prepared by using a densitometer (Biosan, Latvia) and incubated with antibiotics (Ab-i) for 96 h at room temperature. This allows the bacteria sufficient time to grow given their slow growth rate (18 hours doubling time) [30]. Samples without antibiotic incubation (non-incubated (Ab-n)) were kept in the incubator for 96 hours as a control group. In this study Isoniazid (Sigma - Aldrich, USA) was chosen as the antibiotic and was added to the bacteria to the Minimal Inhibitory Concentration (MIC) value of 10 µg/mL. After the incubation time, Ab-n and Ab-i samples were centrifuged at 5,000 rpm

for 5 minutes to pellet cells and the supernatant was discarded and precipitated bacteria was resuspended in isotonic water solution (Daihan, South Korea). This step was repeated three times in order to remove all metabolic impurities. Following this, an equal amount of buffer prepared with 1.08 mL Triton X-100 (Sigma-Aldrich, USA) and 0.045 g Sodium Dodecyl Sulphate (SDS) (Sigma-Aldrich, USA) in 14 mL deionized water was used to bacterial lysis. The Ab-n and Ab-i samples were then gently shaken in the buffer for 10 minutes. The bacterial suspensions were next centrifuged at 10,000 rpm for 10 minutes to remove bacterial debris, and the supernatants were collected. Finally, before performing the electrochemical analysis, the fragmentation process was performed via incubation at 95 °C for 5 minutes [28]. This solution of fragmented oligonucleotides was used for electrochemical detection. It should be noted that in this study specific purification of DNA and/or RNA was not performed from the supernatants. Therefore, the electrochemical measurements were carried out based on hybridization of immobilized probes with both DNA and RNA fragments. Moreover, a non-complementary target of M. smegmatis (used as control measurements) was used to assess specificity. Non-complementary targets also were prepared by the same protocol as TB (Ab-n).

2.2. Detection Method

Electrodes were pretreated by sonicating for 10 minutes while submerged in ethanol, the rinsing in deionized (DI) water for 10 seconds. The cleaning quality of the electrode surface were determined by electrochemical impedance spectroscopy (EIS) and Cyclic voltammetry (CV) techniques (pre-probe measurement). CV and impedance were measured in 1 mM of ferri/ferrocyanide (Sigma-Aldrich, USA) solution in a background of 1xPBS. The various supporting electrolytes are applied in electrochemical sensing such as H2SO4, phosphate buffer (PBS), Britton Rubinson bufer (BR), and KCl [31]. In this study, we utilized PBS as supporting electrolyte of experiments. PBS buffer is usually employed to imitate physiological conditions. Furthermore, the salts which are used to maintain the pH of PBS solutions usually provide much needed ions for electrochemical experiments. After the pre-probe measurement, the electrodes were washed again to prepare them for the immobilization process. The probe solution was prepared by mixing 15 µM Tris(2-

carboxyethyl)phosphine (TCEP, Sigma-Aldrich, USA) and 9 µl of probe DNA specific to the M. tuberculosis 16S rRNA (5'-[ThiC6][SP18] ACCACAAGACATGCATCCCG -3') (BM Lab, Turkey) and kept at room temperature for 1 hour [29]. Following this, the probe solution was dropped on the working electrodes and then incubated at room temperature for 18-24 h [28]. The surfaces incubated with probe solution were washed with DI water for 10 seconds and then incubated with 3-mercapto-1-propanol (MCP-95%, Sigma-Aldrich, USA) solution, which was prepared using 5 mM TCEP and 11.5 mM MCP at room temperature for 1 hour. The post-probe stage was measured using 1 mM ferri/ferrocyanide to evaluate immobilization efficiency. The electrodes were washed with DI water for 10 seconds and dried by nitrogen gas [32]. Finally, the fragmented oligonucleotides were directly dropped onto the electrodes which had been functionalized with probes. The electrodes were incubated for 1 hour at room temperature [33]. Following hybridisation, the electrodes were washed with 5% PBS (Sigma-Aldrich, USA) solution which had been diluted in DI water. The post-target step was again measured by dropping 1 mM ferri/ferrocyanide on the electrodes [32]. Non-complementary experiments were carried out with the same protocol used for the complementary experiments.

Pre-probe, post-probe and post-target electrochemical measurements were carried out in a Faraday Cage using a portable potentiostat device (PalmSens PS4, Houten, the Netherlands). CV and EIS techniques were used in the measurements. The sensitivity of the sensor platform was investigated by applying different initial concentrations (10^8-10^3 CFU/mL) of TB bacteria. Therefore, the TB was cultured at different concentration and lysis as described before. The obtained lysates were centrifuged at 10,000 rpm for 10 min and supernatants were heated at 95° C for 5 min in order to perform fragmentation process. The obtained fragmented solutions were added separately onto the electrodes. EIS measurements were performed after the incubation and washing process, and then normalized Charge Transfer Resistance (Rct) values were calculated. We applied a general linear regression analysis to define the LOD and LOQ parameters represented as 3.3x (σ/s) and 10x (σ/s), respectively [34, 35]; where σ represents the standard deviation and s depicts the slope of the plotted from the Rct vs. concentration of TB.

2.3. Electrochemical Measurement

SPGEs were obtained from Metrohm (Herisau, Switzerland). The electrodes used in this study were a C223AT (high-temperature cure) three-electrode system that contains a silver reference electrode and gold counter and working electrodes. The working electrode area is 1.6 mm and ceramic substrate of screen-printed electrode is L33 x W10 x H0.5 mm.

CV in 1 mM ferri/ferrocyanide solution was used for the characterization of the cleaned electrode surface so that peak current and peak separation could be determined. CV measurements were taken between the potentials -0.25 V and 0.5 V at 0.1 V/s. In addition, EIS measurements were carried out between 100kHz and 0.1Hz frequencies at open circuit in ferri/ferrocyanide measurement buffer. Electrochemical impedance measurements were performed at the open-circuit potential with a 10 mV perturbation and E_{dc} 0.0 V and E_{ac} 0.01 V. In order to specify the Rct fitting was performed using the Randles equivalent circuit.

2.4. Statistical Analysis

SPSS software was applied for statistical analysis. The t-test was used for statistical significance testing. The threshold for significance was set at 0.05 ($p < 0.05$). Error bars depict a standard deviation of the mean (n= 3).

3. Results and Discussion

Figure 1 illustrates the summary of the experiments. Firstly, the prepared TB was added in two different bottles with the same concentration. Then, the antibiotic was added to a bottle to supply the Ab-i sample (Figure 1a). Two samples were kept in the incubator for 96 hours. After the specified time expired, the lysis and fragmentation processes were performed on the samples (Figure 1b). Finally, this solution of fragmented oligonucleotides was dropped onto the immobilized electrodes for electrochemical detection and AST determination (Figure 1c).

Cleaning the electrode surface is an important factor to improve the self-assembled monolayer (SAM) performance[36, 37]. Hence, before starting the measurements, all electrodes were cleaned with ethanol. CV based electrode surface cleaning using H_2SO_4 is known as a common method that is widely used in literature [38, 39]. However, this

technique shows some disadvantages especially in terms of the time. Therefore, in this study cleaning approaches was performed using ethanol [25]. Figure 2a displays a CV from the ferri-ferrocyanide redox reaction on clean electrodes, which can give an indication of the surface quality of an electrode. The peak-to-peak separation value was calculated as 73 mV. According to the Nernst equation, the ideal peak-to-peak separation is 59 mV [38, 39] and the value obtained here is acceptably close to this ideal value. This also demonstrates that the surface of the electrode is suitably clean for SAM formation.

EIS is frequently used in the molecular level detection of analytes due to its sensitivity and label free properties [40, 41]. In this study, EIS analysis was performed on TB extracts using an inexpensive commercially available SPGE [42]. Figure 2b shows the signal change magnitude (100 x post-target / post-probe) of TB after 1 day and 4 days incubation. The signal change magnitudes were approximately 21% on the first day and 157% on the 4th day. The results indicated that performing AST assays on the first day was not appropriate to maximise the signal response. TB AST assays were therefore evaluated on the 4th day (96 hours) post-incubation. In our previous study, we successfully performed AST assays for *Mycobacterium smegmatis* (MSM) after 1 day incubation, due to the much more rapid growth of this organism [29, 43].

To specify the sensitivity of the proposed sensor, we examined EIS changes for different concentrations of TB (10^8-10^3). The results demonstrate that the applied sensor platform detects TBs in the 10^8-10^3 CFU/mL range with a significant trend (Figure 3a). Figure 3b demonstrate calibration curve of all experiments. The Rct values obtained at different concentrations of *M. tuberculosis* were subjected to regression analysis and then the standard deviation (1.3698) was obtained. The slope was calculated as 1.1834 in the calibration graph equation, as shown in Figure 3b. Finally, equations 1 and 2 were applied to obtain LOD and LOQ.

$$\mathrm{LOD} = 3.3 \times \left(\frac{\sigma}{s}\right) \qquad \text{(Equation 1)}$$

$$\mathrm{LOQ} = 10 \times \left(\frac{\sigma}{s}\right) \qquad \text{(Equation 2)}$$

where σ represents the standard deviation and s depicts the slope of the plotted from the Rct vs. concentration of TB.

LOD and LOQ values were obtained as $10^{3.82}$ and $10^{11.59}$ CFU/mL, respectively. In the literature, LOD reported for *M. tuberculosis* as 10^4 CFU/ml by electrical impedance spectroscopy **[44]**.

The AST assay for TB was evaluated with Isoniazid, one of the most established treatments for TB with isoniazid resistant TB being the most common drug resistant form at present [9]. The AST was performed, and susceptibility of TB was electrochemically evaluated through specific hybridization of fragmented DNA/RNA with immobilized oligonucleotide probes. The Rct values have been extracted through Randles equivalent circuit fitting (the Randles equivalent circuit used for data fitting included in Figure 4a). In EIS, impedance behaviour is modelled by solution resistance (Rs), constant phase element (CPE), RCT and Warburg impedance (W), and these are expressed by Randles circuit and Nyquist plot is illustrated by these elements. Although the Randle's circuit typically employs a capacitor, to model the double layer capacitance, it is often necessary to substitute it for CPE to account for more complex electrode surfaces found with SPEs and bio-functionalized electrodes [36]. Figures 4a and 4b show the impedance response following DNA hybridization of Ab-n and Ab-i TB extracts. Impedance values for pre-probe (blue) and post-probe (red) of both experiments are 1.13 ± 0.06 kΩ and 4.46 ± 0.95 kΩ, respectively Immobilization of thiol probes by self-assembly on working electrodes is frequently used in DNA biosensors and fabrication of electrochemical systems [45]. Binding of thiol groups to the gold surface (working electrode) is a result of strong interaction(chemisorption) between the sulfur and gold atoms [46]. An increase in the post-probe Rct indicates successful immobilization of the probes. Following incubation with the target, the post-target impedance values were obtained, and are shown in green. As can be seen in Figure 4a, Ab-n samples were subjected to a high hybridization and the post-target impedance value has increased to 9.95±0.10 kΩ. Ab-n experiments are a culture without the addition of antibiotic. Therefore, the culture grows in the medium when there aren't antibiotics in the media. The Ab-i sample did not exhibit a significant increase in Rct after adding lysed bacteria to the electrode (Figure 4b), indicating the inhibition of the growth of bacteria upon antibiotic incubation. Ab-i is a culture that bacteria were placed

in antibiotic environment. Ab-i contains less DNA/RNA than Ab-n, as concluded from the Figure 4a and Figure 4b. Therefore, Ab-n has a high Rct value in the post-target step, while in Ab-i has a low Rct in the post-target step. Therefore, CFU of Ab-n is higher than Ab-i. This is because of the fact that the susceptible bacteria stop growing upon antibiotic treatment. These results demonstrate that EIS-based analysis for AST evaluation of TB was successfully performed with Isoniazid antibiotic after 4 days of incubation. Furthermore, to confirm our results, we applied MSM bacteria as a non-complementary target. Figure 4c shows that there was no significant signal change in the post-probe Rct when adding lysed MSM bacteria to the electrodes. Therefore, the results indicate that our proposed electrochemical-based sensor platform has high performance and specificity for determining antibiotic susceptibility in TB bacteria within four days. In order to compare the obtained results more clearly, Figure 4d shows the signal change magnitudes of Ab-n, Ab-i, and MSM samples. The signal change magnitude for the Ab-n sample is approximately increased up to 150%, which is statistically significant compared to the other extracts tested. The merit of the analytical performance of the biosensor is shown by its stability and reproducibility. In our study, every experiment was repeated at least 3 times to ensure that the results were both consistent and repeatable. Section 3 included the results obtained from the impedance measurements presented in Figure 2, 3 and 4, which demonstrated the repeatability of the measurements performed by using the modified SPGEs. Both the charge transfer resistances obtained by the fitting of the implemented mathematical model to the impedance response and the corresponding calibration plot showed that experiments ensured the repeatability of the Stability and reproducibility.

In the literature, nucleic acid-based techniques for TB have been reported which could detect up to 120 min such as GenoType Mycobacteria Direct assay (GTMD) [47, 48], quantitative real-time PCR [49], Xpert MTB/RIF [50], Conventional PCR [51] and line probe assays [52]. In this study, the assay successfully validated was carried out in less than 120 min. Currently, the diagnostic methods available for TB detection are not adequately efficient for detecting the disease at an early stage. Furthermore, they do not differentiate between latent and active forms of TB due to insufficient specificity and sensitivity. TB detection methods are mostly limited by the long growth period of TB (minimum of 17 days), the late onset of symptoms (pulmonary TB), and low bacterial load in sputum even in active forms. Electrochemical sensors can accomplish AST test

in short time due to its high specificity for bacterial pathogens in blood culture. 16s rRNA can be detected in real-time and without prior purification [18].

In our previous study, we developed a protocol using oligonucleotide probes for specific detection of DNA target mimicking a section of 16S rDNA from TB [32]. In this study, we applied the developed protocol and successfully detected DNA and RNA sequences from TB bacteria using SPGEs. In the literature, there are several studies detecting TB which use complex electrochemical processes and are therefore often difficult to implement and replicate [53-56], particularly in resource limited settings. In these studies, various different electrode formats have been used to detect TB, including bare gold disk electrodes [53] , graphene or graphene oxide electrodes [42, 44, 56], graphite [54], and a mechanical pencil as a graphite electrode [53]. For example, Hamdan et al. developed an electrochemical biosensor for the detection of TB from the PCR amplified products using pencil graphite electrodes and Methylene Blue [39]. Other groups have used more complex electrode setups including using a biotinylated probe and avidin-attached, poly(L-glutamic) acid coated pencil graphite electrode [57]. The work presented herein demonstrates that SPGEs can be applied as a low-cost electrode for AST determination for TB bacteria by using bespoke genetic probes. This provides a simple, label-free technique to detect TB nucleic acids. Our proposed sensor shows a remarkable improvement in assay time for TB when compared to other non-electrochemical studies in the literature, with some examples achieving an average reporting time of 11 or 12 days, and a lower limit of 6 days [58, 59]. By comparison 96 hours is a very useful time to result with give rapid AST of TB at low cost to widen patient access to drug susceptibility testing and improve outcomes through improved treatment efficacy at an earlier stage of infection. Moreover, different LOD values have been reported in the literature in terms of the DNA probe [60-64] and DNA aptamer [65-68] based sensors. The lowest LOD reported from an electrochemical DNA biosensor was based on graphene-modified iron-oxide chitosan with 0.9 fg/ml [69]. Moreover, Pourmir et al., reported a LOD value for TB 104 CFU/ml [44] while the designed sensor in our study obtained $10^{3.82}$ CFU/mL which is considered as a good value.

The electrochemical biosensors have highest potential for diagnostic TB and its drug resistance. Compare to others diagnostic methods biosensor are fast, valid, and cost-effective. The features of Screen Printed Electrodes (SPEs) show many advantages

compare to traditional electrodes such as ease of fabrication- cleaning procedures, reliability, low-cost, repeatability and provide rapid time to result. SPEs are amenable to mass production at relatively low-cost compared to traditional macro or microelectrodes (< £2 each) [70]. However, SPEs have a very rough surface compared to the electrodes produced by thermal evaporation or sputter deposition method. In our previous work, we reported that the root mean square values are 172.5 and 7.3 nm for the SPEs and the electrode which was fabricated by a thermal evaporation method, respectively [71]. The homogeneous surface of the electrodes which are fabricated by a thermal evaporation method cause an improvement of sensitivity in EIS-based experiments. [71]. Therefore, the type of used electrode in the designed sensor can be considered as a drawback.

4. Conclusions

In this study, we successfully employed label-free molecular-based AST for the case of slow-growing *Mycobacterium tuberculolsis* – a pathogen of high clinical importance which is typically slow and difficult to culture. Electrochemical impedance spectroscopy was used due to its novel advantages such as rapid measurement time, high sensitivity, selectivity, low cost, and simplicity. Specific hybridization of genetic sequences as a proxy for nucleic acid levels and detection thereafter was successfully demonstrated as an effective AST measurement for TB. Electrochemical measurements demonstrated that the rapid, qualitative analysis of such slow-growing bacteria was possible in 4 days.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

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Figure Legends

Figure 1. a) Preparation of Ab-i and Ab-n samples; *M. tuberculosis* was incubated with or without isoniazid for 96 hours b) Lysis and fragmentation of bacteria; Bacteria were washed by centrifugation, chemically lysed and the nucleic acids isolated and fragmented using high temperature. c) Electrochemical experiment process; electrodes functionalized with DNA probe and backfilled with MCP were incubated with fragmented nucleic acids for hybridization.

Figure 2. a) CV voltammogram of clean screen-printed gold electrode. b) Mean EIS signal changes after exposure to TB following 1 or 4 days incubation without antibiotic.

Figure 3. a) EIS responses for different concentration and b) corresponding normalized Rct values and calibration curves for different TB concentrations.

Figure 4. EIS signal responses for electrodes before and after exposure to extracts of (a) antibiotic free (Ab-n) M. tuberculosis, (b) antibiotic incubated (Ab-i) M. tuberculosis, (c) non-complementary M. smegmatis. d) Mean EIS signal changes following incubation of the extracts in (a), (b) and (c).