UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF SANDWICH-TYPE ELECTROCHEMICAL ANTIBODY AND APTAMER SENSORS FOR DETECTION OF Mycobacterium tuberculosis

UMI ZULAIKHA BINTI MOHD AZMI

ITMA 2021 4

DEVELOPMENT OF SANDWICH-TYPE ELECTROCHEMICAL ANTIBODY AND APTAMER SENSORS FOR DETECTION OF *Mycobacterium tuberculosis*

By

UMI ZULAIKHA BINTI MOHD AZMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2020

DEDICATION

Alhamdulillah thank you Allah for giving me the opportunity to finish writing this thesis. Special thanks to my beloved supervisor for her continuous support from day one until I manage to complete doing my research. Also to my parents and parents-in-law, for their endless support and prayers throughout my journey. This thesis is wholeheartedly dedicated to my lovely husband, Muhammad Zamiruddin bin Sahar for his patience and encouragement which make me able to achieve the success. I would also like to thank my siblings and siblings-in-law for their concern about all my problems during this process. Last but not least, to all my dearest friends especially those who are very close to me, Suhainie, Fariza Aina, Nazifah, Nor Hidayat, Norzila, Ain Shahera, Fatimah, Nur Hidayah, Zurmira, Siti Arisyah, Noremylia, Faezah, Siti Khadijah, Siti Fatimah, Nor Zida, Hamizah, Zulaiha, Syahira, Hayati, Putri, Nurul Asyikeen, Syahirah, Shahirah, Chong, Zarif, Zul, Chin and many more who always stay positive and give their support.

Thank you so much, you all inspired me to do my best.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT OF SANDWICH-TYPE ELECTROCHEMICAL ANTIBODY AND APTAMER SENSORS FOR DETECTION OF *Mycobacterium tuberculosis*

By

UMI ZULAIKHA BINTI MOHD AZMI

December 2020

Chair : Nor Azah binti Yusof, PhD
Institute : Advanced Technology

Early diagnosis of *Mycobacterium tuberculosis* is very important to reduce the number of fatal cases and allow fast recovery. This can be done by using smear microscopy method; however, the interpretation of results requires skilled personnel due to tendency of the method to produce false-negative results (lack of sensitivity). Therefore, in this study, sandwich-type electrochemical immunosensor and aptasensor were compared for detection of CFP10-ESAT6 complex antigen as a biomarker of *Mycobacterium tuberculosis*, based on graphene/polyaniline (GP/PANI) modified screen-printed gold electrode (SPGE). GP/PANI nanocomposite was synthesized and characterized using Raman spectroscopy and Field Emission Scanning Electron Microscope (FESEM) in order to confirm the formation of nanocomposite. Then, it was dispersed in 3-aminopropyltriethoxysilane (APTES) and used as an electrode modifier. The morphology of the fabricated GP/PANI-SPGE was analyzed using FESEM and it showed a rough and porous surface while Energy Dispersive X-ray (EDX) spectroscopy has shown all the presence elements of GP/PANI on SPGE surface. Based on cyclic voltammetry (CV) characterization, the fabricated GP/PANI-SPGE has shown a large surface area compared to unmodified electrode. Capturing probes (anti-CFP10-ESAT6 antibodies or aptamer) were immobilized onto the surface of GP/PANI-SPGE. Iron/gold magnetic nanoparticles (Fe_3O_4/Au MNPs) conjugated with anti-CFP10-ESAT6 was used to complete the sandwich system. The Fe_3O_4/Au MNPs was synthesized and characterized using Ultraviolet-Visible (UV-Vis) spectrophotometer, High Resolution-Transmission Electron Microscopy (HR-TEM) and X-ray Diffraction (XRD) before conjugating it with anti-CFP10-ESAT6. Differential pulse voltammetry (DPV) technique was used to investigate the analytical performance of both immunosensor and aptasensor with its corresponding CFP10-ESAT6 antigen. The detection time

was within 2 hours. Under optimum conditions, both sensors showed comparable results with almost the same limit of detection (LOD) of 1.47 ng/mL for immunosensor and 1.52 ng/mL for aptasensor. However, aptasensor showed better specificity and reproducibility than immunosensor. The methods developed from these processes were then integrated into a portable reader, which provides a good correlation with conventional methods on detection of *M. tuberculosis* in sputum samples. Henceforth, the developed biosensor demonstrated potential as a practical screening tool for *M. tuberculosis* detection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN ELEKTROKIMIA SENSOR JENIS SANDWIC BERDASARKAN ANTIBODI DAN APTAMER BAGI PENGESANAN *Mycobacterium tuberculosis*

Oleh

UMI ZULAIKHA BINTI MOHD AZMI

Disember 2020

Pengerusi : Nor Azah binti Yusof, PhD
Institut : Teknologi Maju

Pengesanan awal *Mycobacterium tuberculosis* adalah penting untuk mengurangkan bilangan kes kematian dan mempercepatkan kesembuhan. Hal ini boleh dilaksanakan dengan menggunakan teknik mikroskopik bagi sapuan (*smear*); walau bagaimanapun, tafsiran hasilnya memerlukan kakitangan yang mahir kerana kaedah tersebut cenderung untuk menghasilkan keputusan negatif-palsu (kurang kepekaan). Oleh itu, dalam kajian ini, elektrokimia *immunosensor* dan *aptasensor* jenis sandwic berdasarkan elektrod emas bercetak skrin (SPGE) modifikasi grafin/polianilin (GP/PANI) telah dibandingkan untuk pengesanan antigen kompleks CFP10-ESAT6 sebagai penanda bio *Mycobacterium tuberculosis* dibentangkan. GP/PANI nanokomposit telah disintesis dan dicirikan menggunakan spektroskopi Raman dan FESEM untuk mengesahkan nanokomposit yang terbentuk. Selepas itu, ia disebarkan di dalam APTES dan digunakan sebagai pengubah suai elektrod. Morfologi fabrikasi GP/PANI-SPGE telah dianalisis menggunakan FESEM dan menunjukkan permukaan yang kasar dan berliang manakala spektroskopi EDX mempamerkan semua elemen GP/PANI yang hadir di atas permukaan SPGE. Berdasarkan pencirian CV, fabrikasi GP/PANI-SPGE menunjukkan luas permukaan yang besar berbanding dengan elektrod yang tidak dimodifikasikan. Prob penangkap (antibodi CFP10-ESAT6 atau *aptamer*) telah dilekatkan di atas permukaan GP/PANI-SPGE. Nanopartikel magnet besi/emas (Fe_3O_4/Au MNPs) yang dikonjugasikan dengan anti-CFP10-ESAT6 telah digunakan untuk melengkapkan sistem sandwic. Fe_3O_4/Au MNPs telah disintesis dan dicirikan dengan menggunakan spektrofotometer UV-Vis, HR-TEM dan XRD sebelum dikonjugasikan dengan anti-CFP10-ESAT6. Teknik voltammetri nadi pembezaan (DPV) telah digunakan untuk mengkaji prestasi analisis *immunosensor* dan *aptasensor* dengan antigen CFP10-ESAT6. Masa pengesanan adalah 2 jam. Dalam keadaan optimum, kedua-dua sensor menunjukkan hasil yang setanding

dengan had pengesanan yang hampir sama iaitu 1.47 ng/mL untuk *immunosensor* dan 1.52 ng/mL untuk *aptasensor*. Walau bagaimanapun, *aptasensor* menunjukkan kekhususan dan kebolehulangan yang lebih baik berbanding dengan *imunosensor*. Kaedah yang dikembangkan dari proses ini kemudian disatukan ke dalam pembaca mudah alih yang memberikan korelasi yang baik dengan kaedah konvensional dalam pengesanan *Mycobacterium tuberculosis* pada sampel kahak. Seterusnya, peranti menunjukkan potensi sebagai sebagai alat saringan yang lebih praktikal.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and Merciful, first and foremost, Alhamdulillah for His blessings in giving me the chance to complete my research and thesis. With His guidance and help, this project runs smoothly according to the plan. Special appreciation and deepest gratitude go to my supervisor, Prof Dr Nor Azah Yusof for her valuable knowledge and continuous support for this study. Her guidance helped me a lot in all time of research and writing of this thesis. Besides that, thank you to my co-supervisors, Assoc. Prof. Dr Jaafar Abdullah, Dr Shahrul Ainliah Alang Ahmad and Dr Nurul Hanun Ahmad Raston, for their encouragements and valuable comments on my PhD work.

My special thanks also goes to Assoc. Prof. Dr. Siti Suraiya Md Noor from Department of Microbiology and Parasitology, Hospital Universiti Sains Malaysia (HUSM) for providing me a number of samples for clinical application. Thank you also to the laboratory staff, Encik Ali and his colleagues, for helping me to prepare the sputum samples and guiding me as well.

My sincerest appreciation also goes to my family and lab mates (BASL103) for their support and advise until I successfully finish my work and thesis as well. Next, thanks to all staff from Institute of Advanced Technology, UPM for their concern and help upon handling the instrumentation.

Last but not least, I would like to acknowledge my financial support throughout my three-years study, Graduate Research Fellowship (GRF), UPM. Besides, thank you also to Nanomalaysia Berhad and UPM for their research fund which help me a lot to finish my work.

Thank you very much.

UMI ZULAIKHA BINTI MOHD AZMI

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Nor Azah Binti Yusof, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Jaafar Bin Abdullah, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Shahrul Ainliah Alang Ahmad, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Nurul Hanun Ahmad Raston, PhD
Senior lecturer
Department of Biological Sciences and Biotechnology,
Faculty of Science
Universiti Kebangsaan Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 June 2021

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ab	Primary antibody
AuNPs	Gold Nanoparticles
CapAb	Capture antibody
CapApt	Capture aptamer
CD	Circular Dichroism
DNA	Deoxyribonucleic acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
ELISA	Enzyme Linked Immunosorbent Assay
Fe_3O_4/Au MNPs	Iron/gold Magnetic Nanoparticles
GP	Graphene
GU	Growth Unit
LED-FM	Light Emitting Diode-based Fluorescence Microscopy
M. tuberculosis	*Mycobacterium tuberculosis*
MGIT	Mycobacteria Growth Indicator Tube
MTBC	*M. tuberculosis* complex
NHS	N-hydroxysuccinimide
PANI	Polyaniline
POC	Point-of-care
SPE	Screen-Printed Electrodes
SPGE	Screen Printed Gold Electrode
SPR	Surface Plasmon Resonance
TB	Tuberculosis
WHO	World Health Organization

HIV	Human Immunodeficiency Virus
IUPAC	International Union of Pure and Applied Chemistry
POCT	Point-of-care testing
Igs	Immunoglobulins
SELEX	Systematic Evolution of Ligands by Exponential Enrichment
PCR	Polymerase Chain Reaction
ssDNA	Single stranded DNA
APTES	3-aminopropyltriethoxysilane
GOx	Glucose oxidase
rGO	Reduced graphene oxide
LOD	Limit of detection
HRP	Horseradish peroxidase
H_2O_2	Hydrogen peroxide
IFN-γ	Interferon-γ
NPs	Nanoparticles
AgNPs	Silver nanoparticles
PBS	Phosphate-buffered saline
BCG	Bacillus Calmette-Guérin

CHAPTER 1

INTRODUCTION

1.1 Background of study

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) pathogen is one of the largest causes of death after human immunodeficiency virus (HIV). Other than lungs, this bacterium can actively spread to other parts of the body when the immune system is weak. This contagious disease is classified as airborne because it can be transmitted to other people when the infected person expels the bacteria into the air through coughing, sneezing, or even talking (Ariffin et al., 2020). Globally, the number of TB reported cases increased from 9.6 million in 2014 to 10 million in 2019 (including deaths) (WHO, 2015, 2019). In Malaysia, number of TB cases is high with a current estimated incidence of 92 cases per 100,000 populations in 2019 due to high influx of illegal immigration, HIV, drug resistance, delayed diagnosis, high smoking rates, and diabetes (Mat Zaid et al., 2017; Mohd Bakhori et al., 2020; WHO, 2020). Therefore, early and accurate detection of TB will be a good initiative to isolate the patients and control the disease.

Several conventional methods that are commonly used for active TB detection such as sputum smear microscopy and culture of *M. tuberculosis* from patient samples. However, these conventional methods are time-consuming, lack of sensitivity and specificity, unreliable and tend to give false result, especially for individuals with HIV co-infection (Ariffin et al., 2020; Mohd Bakhori et al., 2020; Tufa et al., 2018). Hence, the development of rapid and accurate diagnostic tools are essential to improve TB control in developing countries such as fluorescence microscopy, mycobacteria growth indicator tube (MGIT) and biosensors (Golichenari et al., 2019; Srivastava et al., 2016). These methods have been applied for different samples such as urine (Kim et al., 2017; Mukundan et al., 2012; Phan et al., 2018), sputum (Kim et al., 2017; Liu et al., 2014; Mohd Bakhori et al., 2018) and blood (Li et al., 2018). Among these methods, biosensors have tremendously developed due to high sensitivity, low-cost and effective for detection of various pathogens (Chan et al., 2016; Li et al., 2018).

Biosensor can be generally defined as a device that consists of a biological recognition system (aptamers, proteins, antibodies, enzymes) and coupled to a transducer for signal processing, to detect and quantify a particular analyte. Biosensors provide advanced platforms for biomarker analysis with a number of advantages such as user-friendly, rapid and robust as well as offering multianalyte testing capability (Srivastava et al., 2016); however, a specific biomarker is necessary in order to detect a disease accurately (Phan et al., 2018). Immunosensors or antibody-based sensors are based on antibody to capture the target antigen (Felix & Angnes, 2018), while aptamer-based sensors or known as aptasensors are based on nucleic acid to identify various

targets such as proteins, peptides and amino acids (Kim et al., 2016). These biosensors are core developments in clinical diagnosis especially in the detection of *M. tuberculosis* for TB disease (Ansari et al., 2018; Ariffin et al., 2020; Sypabekova et al., 2019). This is due to the highly specific molecular recognition of antibody or aptamer with antigen and high sensitivity of the sensor (Chan et al., 2016; Wei et al., 2010).

Electrochemical biosensor offers high sensitivity, simplicity and easily miniaturize, which made the electrochemical biosensor among the top choices in biosensing platform (Golichenari et al., 2019; Tufa et al., 2018). Generally, electrochemical detection can be performed either by labelling or without labelling. Label-based or sandwich-type electrochemical biosensor consists of two biorecognition elements used to anchor the target biomarker; (i) capture probe and (ii) detection probe, unlike label-free electrochemical biosensor (Kokkinos et al., 2016). According to this principle, the specific binding between target biomarkers and the immobilized biomolecules on the electrode, such as antigen-antibody, DNA-DNA and antigen-aptamer, has been extensively used to capture analytes or probes to improve the selectivity of the sensors. Meanwhile, the enhancement of detection sensitivity can be achieved by conjugating nanomaterials with biorecognition element, known as detection probe (Zhang et al., 2016). It is also important to ensure that the sensors are capable to detect a very low amount of analytes.

The utilization of nanomaterials in the development of biosensors has been frequently used in order to enhance the performance. Nanomaterials are proven can increase the rate of electron transfer as well as improve the electrochemical signals as they have good conductivity and electrocatalytic effect (Cho et al., 2018). In addition, the usage of nanomaterials in modifying working electrodes provides more roughened surfaces that facilitate the attachment of the biological elements to electrode surfaces. Apart from that, nanomaterials can increase the electroactive surface area; hence, leads to better sensitivity and reduce the detection limits. Different nanomaterials have been used in biosensors, including, carbon nanomaterials, conducting polymers, magnetic nanoparticles or magnetic beads (MBs), gold nanoparticles and nanocomposites (Chen et al., 2007; Li et al., 2018; Liu et al., 2014; Zhang et al., 2016).

1.2 Problem statements

The common method used for diagnosing *M. tuberculosis* is sputum smear microscopy (Mukundan et al., 2012). Sputum is a thick fluid that is produced in the lungs and the airways leading to the lungs. Sputum sample is typically collected from an infected person who has cough (Mat Zaid et al., 2017). Bacteria in the sputum sample are then examined under a microscope. Although this method is simple and inexpensive, it is insensitive and unable to identify half of the positive TB infections (Kim et al., 2013; Liu et al., 2014; Torati et al., 2016). Another method called culture method is a 'gold standard' in determining *M. tuberculosis*. However, this method requires 4 – 8 weeks to

grow the mycobacterium. Besides these methods, chest x-ray, tuberculin skin test, fluorescent microscopy and serological test have been used due to their high accuracy. Nevertheless, these methods are time-consuming, require expensive instrumentation and some of the techniques are unsuitable for active TB detection (Mat Zaid et al., 2017).

In order to overcome these issues, electrochemical biosensors based on antibodies and aptamer are recently developed as a promising way for antigen detection in clinical diagnosis including *M. tuberculosis*. These types of biosensors provide several advantages such as high specificity, low detection limit, requires small amount of analytes and can be easily miniaturized (Butmee et al., 2020; Golichenari et al., 2019). However, electrochemical detection of *M. tuberculosis* antigen at an early stage is very challenging because the growth is slow in real samples. Therefore, the selection of biomarkers, which are secreted earlier such as CFP10-ESAT6 complex is essential to aid the detection of the bacteria at an early stage (Felix & Angnes, 2018; Mohd Bakhori et al., 2020).

The electrode in an electrochemical biosensor provides a solid support for the immobilization of the biorecognition element (i.e., antibody or aptamer) as well as a sensing means for the electrons produced from the biological reaction (Cho et al., 2018). Therefore, the selection of electrode combination with a proper surface modification is crucial to provide an effective electrochemical performance. The incorporation of screen-printed electrodes (SPE) with nanomaterials can be used to improve the electrochemical properties. Graphene (GP) in the form of a two-dimensional honeycomb has a lot of useful properties such as a large surface area and numerous active sites. GP is superior than other carbon allotropes based on the following properties; high electron transfer speed, high thermal conductivity, good mechanical flexibility and biocompatible (Li et al., 2015; Roberts et al., 2019). Meanwhile, polyaniline (PANI) is a conducting polymer that has been widely used because of its easy fabrication process and inexpensive. In electrochemical sensors, PANI is reported to have fast electron transfer and excellent electrochemical activity (Shoaie et al., 2019). Therefore, GP and PANI is a highly potential combination for our proposed electrochemical biosensors to immobilize the capture probes.

For detection probe, the core/shell iron oxide/gold magnetic nanoparticles (Fe_3O_4/Au MNPs) were used in this study as a label to conjugate with primary antibody (Ab). Fe_3O_4 MNPs are frequently used to isolate the target molecules from sample solution by the application of an external magnetic field. However, magnetic nanoparticles tend to lose their magnetic properties when applied in complex biological systems (Silva et al., 2016). Gold nanoparticles (AuNPs) have the ability to protect Fe_3O_4 MNPs from oxidize as well as able to functionalize through Au-S binding (Freitas et al., 2014). Despite, AuNPs require a centrifugation step to separate the target molecules from the sample solution, which is time-consuming. Thus, the formation of Fe_3O_4 MNPs as a core and AuNPs as a shell are proposed in this study.

1.3 Novelty of research

In this study, the utilization of sandwich-type electrochemical biosensor involving the immobilization of specific biorecognition elements (e.g. antibody and aptamer) onto GP/PANI nanocomposite modified electrode as a capture probe and Fe_3O_4/Au MNPs as a label for detection of *M. tuberculosis* is the first to be reported. The binding of CapAb-CFP10-ESAT6 antigen was analyzed using enzyme-linked immunosorbent assay (ELISA) technique, while CapApt-CFP10-ESAT6 antigen was evaluated using surface plasmon resonance (SPR) and circular dichroism (CD) technique prior to assays testing. For the immunosensor, a mixture of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride / N-hydroxysuccinimide (EDC/NHS) was used to bind the amine group of GP/PANI surface with carboxyl group of CapAb, while for aptasensor, the GP/PANI surface was crosslinked with CapApt using glutaraldehyde due to the presence of amine group in GP/PANI surface and CapApt. The optimized bioassays were further integrated into a portable reader and applied on real samples. Compared with existing methods, the use of portable electrochemical biosensor represents the most practical and promising technique that could meet the demands of on-site and remote areas point-of-care (POC) diagnostic for *M. tuberculosis* detection. Unlike DNA-based biosensor, both immunosensor and aptasensor do not require the sample to be extracted and thus, reduce the time to obtain the result. Moreover, this non-invasive system which is sputum-based detection could provide an affordable diagnostic test in developing countries.

1.4 Objectives of research

The main aim of the research is to develop novel, ultrasensitive, non-invasive and affordable electrochemical biosensors for the detection of *M. tuberculosis*. The explicit objectives of this project are listed below:

1. To prepare and functionalize core/shell Fe_3O_4/Au MNPs as a label for primary antibody;

2. To prepare GP/PANI nanocomposites and optimize the modification of electrode for immunosensor and aptasensor for electrochemical detection of *M. tuberculosis*;

3. To characterize the fabricated immunosensor and aptasensor for detection of *M. tuberculosis* through electrochemical technique;

4. To evaluate the optimized parameters of developed immunosensor and aptasensor into a portable reader and apply on clinical sputum samples.

1.5 Scope and limitation

In this present study, the biorecognition elements used are specific towards biomarker CFP10-ESAT6 antigen as it has high affinity with the active binding site of antibody and aptamer. Apart from that, the Fe_3O_4/Au MNPs conjugated with primary antibody as detection probe provided better sensitivity for both sensors (immuno and aptamer-based).

However, this study is limited to single biomarker, which is CFP10-ESAT6 antigen. Furthermore, the application of portable sensors on real samples is limited due to the limited access for samples from hospital and clinic. Besides that, the fabricated modified electrode is not stable for transportation and requires proper packaging for on-site real sample testing.

REFERENCES

Abd Rashid, J. I., Yusof, N. A., Abdullah, J., Hashim, U., & Hajian, R. (2015). A Novel Disposable Biosensor Based on SiNWs/AuNPs Modified-Screen Printed Electrode for Dengue Virus DNA Oligomer Detection. *IEEE Sensors Journal*, *15*(8), 4420–4427. https://doi.org/10.1109/JSEN.2015.2417911

Abdul Rashid, J. I., Yusof, N. A., Abdullah, J., Hashim, U., & Hajian, R. (2014). The utilization of SiNWs/AuNPs-modified indium tin oxide (ITO) in fabrication of electrochemical DNA sensor. *Materials Science and Engineering C*, *45*, 270–276. https://doi.org/10.1016/j.msec.2014.09.010

Ahmad Raston, N. H., Nguyen, V. T., & Gu, M. B. (2017). A new lateral flow strip assay (LFSA) using a pair of aptamers for the detection of Vaspin. *Biosensors and Bioelectronics*, *93*(November 2016), 21–25. https://doi.org/10.1016/j.bios.2016.11.061

Ansari, N., Ghazvini, K., Ramezani, M., Shahdordizadeh, M., Yazdian-Robati, R., Abnous, K., & Taghdisi, S. M. (2018). Selection of DNA aptamers against Mycobacterium tuberculosis Ag85A, and its application in a graphene oxide-based fluorometric assay. *Microchimica Acta*, *185*(21), 1–8. https://doi.org/10.1007/s00604-017-2550-3

Ariffin, N., Yusof, N. A., Abdullah, J., Abd Rahman, S. F., Ahmad Raston, N. H., Kusnin, N., & Suraiya, S. (2020). Lateral flow immunoassay for naked eye detection of Mycobacterium tuberculosis. *Journal of Sensors*, *2020*(Article ID 1365983), 1–10. https://doi.org/10.1155/2020/1365983

Arifin, N. F. T., Zulkipli, N. A. N., Yusof, N., Ismail, A. F., Aziz, F., Wan Salleh, W. N., Jaafar, J., Nordin, N. A. H. M., & Sazali, N. (2019). Preparation and characterization of APTES-functionalized graphene oxide for CO2 adsorption. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, *61*(2), 297–305. https://doi.org/10.13140/RG.2.2.10612.30085

Azri, F. A., Eissa, S., Zourob, M., Chinnappan, R., Sukor, R., Yusof, N. A., Raston, N. H. A., Alhoshani, A., & Jinap, S. (2020). Electrochemical determination of zearalenone using a label-free competitive aptasensor. *Microchimica Acta*, *187*(266), 1–10. https://doi.org/10.1007/s00604-020-4218-7

Bai, L., Chen, Y., Bai, Y., Chen, Y., Zhou, J., & Huang, A. (2017). Fullerene-doped polyaniline as new redox nanoprobe and catalyst in electrochemical aptasensor for ultrasensitive detection of Mycobacterium tuberculosis MPT64 antigen in human serum. *Biomaterials*, *133*, 11–19. https://doi.org/10.1016/j.biomaterials.2017.04.010

Bai, L., Chen, Y., Liu, X., Zhou, J., Cao, J., Hou, L., & Guo, S. (2019). Ultrasensitive electrochemical detection of Mycobacterium tuberculosis

IS6110 fragment using gold nanoparticles decorated fullerene nanoparticles/nitrogen-doped graphene nanosheet as signal tags. *Analytica Chimica Acta*, *1080*, 75–83. https://doi.org/10.1016/j.aca.2019.06.043

Bo, Y., Yang, H., Hu, Y., Yao, T., & Huang, S. (2011). A novel electrochemical DNA biosensor based on graphene and polyaniline nanowires. *Electrochimica Acta*, *56*, 2676–2681. https://doi.org/10.1016/j.electacta.2010.12.034

Butmee, P., Tumcharern, G., Thouand, G., Kalcher, K., & Samphao, A. (2020). An ultrasensitive immunosensor based on manganese dioxide-graphene nanoplatelets and core shell Fe3O4@Au nanoparticles for label-free detection of carcinoembryonic antigen. *Bioelectrochemistry*, *132*, 107452. https://doi.org/10.1016/j.bioelechem.2019.107452

Caulfield, A. J., & Wengenack, N. L. (2016). Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *4*, 33–43. https://doi.org/10.1016/j.jctube.2016.05.005

Chan, K. F., Lim, H. N., Shams, N., Jayabal, S., Pandikumar, A., & Huang, N. M. (2016). Fabrication of graphene/gold-modified screen-printed electrode for detection of carcinoembryonic antigen. *Materials Science and Engineering C*, *58*, 666–674. https://doi.org/10.1016/j.msec.2015.09.010

Chauhan, R., & Basu, T. (2015). Functionalised Au Coated Iron Oxide Nanocomposites Based Reusable Immunosensor for AFB1 Detection. *Journal of Nanomaterials*, *2015*, 1–16. https://doi.org/10.1155/2015/607268

Chen, Y., Guo, S., Zhao, M., Zhang, P., Xin, Z., Tao, J., & Bai, L. (2018). Amperometric DNA biosensor for Mycobacterium tuberculosis detection using flower-like carbon nanotubes-polyaniline nanohybrid and enzyme-assisted signal amplification strategy. *Biosensors and Bioelectronics*, *119*(June), 215–220. https://doi.org/10.1016/j.bios.2018.08.023

Chen, Y., Liu, X., Guo, S., Cao, J., Zhou, J., Zuo, J., & Bai, L. (2019). A sandwich-type electrochemical aptasensor for Mycobacterium tuberculosis MPT64 antigen detection using C60NPs decorated N-CNTs/GO nanocomposite coupled with conductive PEI-functionalized metal-organic framework. *Biomaterials*, *216*(June), 119253. https://doi.org/10.1016/j.biomaterials.2019.119253

Chen, Z. P., Peng, Z. F., Zhang, P., Jin, X. F., Jiang, J. H., Zhang, X. B., Shen, G. L., & Yu, R. Q. (2007). A sensitive immunosensor using colloidal gold as electrochemical label. *Talanta*, *72*(5), 1800–1804. https://doi.org/10.1016/j.talanta.2007.02.020

Cho, I. H., Lee, J., Kim, J., Kang, M. S., Paik, J. K., Ku, S., Cho, H. M., Irudayaraj, J., & Kim, D. H. (2018). Current technologies of electrochemical immunosensors: Perspective on signal amplification.

Sensors, *18*(207), 1–18. https://doi.org/10.3390/s18010207

Chutichetpong, P., Cheeveewattanagul, N., Srilohasin, P., Rijiravanich, P., Chaiprasert, A., & Surareungchai, W. (2018). Rapid screening drug susceptibility test in tuberculosis using sandwich electrochemical immunosensor. *Analytica Chimica Acta*, *1025*, 108–117. https://doi.org/10.1016/j.aca.2018.04.064

Crick, F. H. C., & Watson, J. D. (1953). Molecular structure of nucleic acids: A structure of deoxyribose nucleic acid. *Nature*, *171*, 737–738.

Cristea, C., Florea, A., Tertiș, M., & Săndulescu, R. (2015). Immunosensors. In T. Rinken (Ed.), *Biosensors - Micro and Nanoscale Applications* (pp. 165–201). IntechOpen. https://doi.org/http://dx.doi.org/10.5772/60524

Cui, Y.-R., Hong, C., Zhou, Y.-L., Li, Y., Gao, X.-M., & Zhang, X.-X. (2011). Synthesis of orientedly bioconjugated core/shell Fe3O4@Au magnetic nanoparticles for cell separation. *Talanta*, *85*, 1246–1252. https://doi.org/10.1016/j.talanta.2011.05.010

Díaz-González, M., González-García, M. B., & Costa-García, A. (2005). Immunosensor for Mycobacterium tuberculosis on screen-printed carbon electrodes. *Biosensors and Bioelectronics*, *20*, 2035–2043. https://doi.org/10.1016/j.bios.2004.09.035

Diouani, M. F., Ouerghi, O., Refai, A., Belgacem, K., Tlili, C., Laouini, D., & Essafi, M. (2017). Detection of ESAT-6 by a label free miniature immuno-electrochemical biosensor as a diagnostic tool for tuberculosis. *Materials Science and Engineering C*, *74*, 465–470.

Duangkaew, P., Tapaneeyakorn, S., Apiwat, C., & Dharakul, T. (2015). Ultrasensitive electrochemical immunosensor based on dual signal ampli fi cation process for p16 INK4a cervical cancer detection in clinical samples. *Biosensors and Bioelectronic*, *74*, 673–679. https://doi.org/10.1016/j.bios.2015.07.004

Ellington, A. D., & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature*, *346*, 818–822. https://doi.org/10.1038/346818a0

Eriksson, K., Palmgren, P., Nyholm, L., & Oscarsson, S. (2012). Electrochemical synthesis of gold and protein gradients on particle surfaces. *Langmuir*, *28*, 10318–10323. https://doi.org/10.1021/la3017694

Fan, W., Zhang, C., Tjiu, W. W., Pramoda, K. P., He, C., & Liu, T. (2013). Graphene-wrapped polyaniline hollow spheres as novel hybrid electrode materials for supercapacitor applications. *ACS Applied Materials & Interfaces*, *5*(8), 3382–3391. https://doi.org/https://doi.org/10.1021/am4003827

Felix, F. S., & Angnes, L. (2018). Electrochemical immunosensors – A powerful tool for analytical applications. *Biosensors and Bioelectronics*, *102*, 470–

478. https://doi.org/10.1016/j.bios.2017.11.029

Freitas, M., Viswanathan, S., Nouws, H. P. A., Oliveira, M. B. P. P., & Delerue-Matos, C. (2014). Iron oxide/gold core/shell nanomagnetic probes and CdS biolabels for amplified electrochemical immunosensing of Salmonella typhimurium. *Biosensors and Bioelectronics*, *51*, 195–200. https://doi.org/10.1016/j.bios.2013.07.048

Goletti, D., Petruccioli, E., Joosten, S. A., & Ottenhoff, T. H. M. (2016). Tuberculosis biomarkers: From diagnosis to protection. *Infectious Disease Reports*, *8*(2), 24–32. https://doi.org/10.4081/idr.2016.6568

Golichenari, B., Nosrati, R., Farokhi-Fard, A., Faal Maleki, M., Gheibi Hayat, S. M., Ghazvini, K., Vaziri, F., & Behravan, J. (2019). Electrochemical-based biosensors for detection of Mycobacterium tuberculosis and tuberculosis biomarkers. *Critical Reviews in Biotechnology*, *39*(8), 1056–1077. https://doi.org/10.1080/07388551.2019.1668348

Golichenari, B., Velonia, K., Nosrati, R., Nezami, A., Farokhi-Fard, A., Abnous, K., Behravan, J., & Tsatsakis, A. M. (2018). Label-free nano-biosensing on the road to tuberculosis detection. *Biosensors and Bioelectronics*, *113*(April), 124–135. https://doi.org/10.1016/j.bios.2018.04.059

Harper, A., & Anderson, M. R. (2010). Electrochemical glucose sensors-developments using electrostatic assembly and carbon nanotubes for biosensor construction. *Sensors*, *10*(9), 8248–8274. https://doi.org/10.3390/s100908248

He, F., Xiong, Y., Liu, J., Tong, F., & Yan, D. (2016). Construction of Au-IDE/CFP10-ESAT6 aptamer/DNA-AuNPs MSPQC for rapid detection of Mycobacterium tuberculosis. *Biosensors and Bioelectronics*, *77*, 799–804. https://doi.org/10.1016/j.bios.2015.10.054

Idegami, K., Chikae, M., Kerman, K., Nagatani, N., Yuhi, T., Endo, T., & Tamiya, E. (2008). Gold nanoparticle-based redox signal enhancement for sensitive detection of human chorionic gonadotropin hormone. *Electroanalysis*, *20*(1), 14–21. https://doi.org/10.1002/elan.200704011

Jaroenram, W., Kampeera, J., Arunrut, N., Karuwan, C., Sappat, A., Khumwan, P., Jaitrong, S., Boonnak, K., Prammananan, T., Chaiprasert, A., Tuantranont, A., & Kiatpathomchai, W. (2020). Graphene-based electrochemical genosensor incorporated loop-mediated isothermal amplification for rapid on-site detection of Mycobacterium tuberculosis. *Journal of Pharmaceutical and Biomedical Analysis*, *186*, 113333. https://doi.org/10.1016/j.jpba.2020.113333

Kerman, K., Morita, Y., Takamura, Y., Ozsoz, M., & Tamiya, E. (2004). Modification of Escherichia coli single-stranded DNA binding protein with gold nanoparticles for electrochemical detection of DNA hybridization. *Analytica Chimica Acta*, *510*, 169–174. https://doi.org/10.1016/j.aca.2003.12.067

Khanmohammadi, A., Aghaie, A., Vahedi, E., Qazvini, A., Ghanei, M., Afkhami, A., Hajian, A., & Bagheri, H. (2020). Electrochemical biosensors for the detection of lung cancer biomarkers: A review. *Talanta*, *206*, 120251. https://doi.org/10.1016/j.talanta.2019.120251

Kim, E. J., Kim, E. B., Lee, S. W., Cheon, S. A., Kim, H. J., Lee, J., Lee, M. K., Ko, S., & Park, T. J. (2017). An easy and sensitive sandwich assay for detection of Mycobacterium tuberculosis Ag85B antigen using quantum dots and gold nanorods. *Biosensors and Bioelectronics*, *87*, 150–156. https://doi.org/10.1016/j.bios.2016.08.034

Kim, J., Lee, J., Lee, K. I., Park, T. J., Kim, H. J., & Lee, J. (2013). Rapid monitoring of CFP-10 during culture of Mycobacterium tuberculosis by using a magnetophoretic immunoassay. *Sensors and Actuators B: Chemical*, *177*, 327–333. https://doi.org/10.1016/j.snb.2012.11.011

Kim, J., Lee, K. S., Kim, E. B., Paik, S., Chang, C. L., Park, T. J., Kim, H. J., & Lee, J. (2017). Early detection of the growth of Mycobacterium tuberculosis using magnetophoretic immunoassay in liquid culture. *Biosensors and Bioelectronics*, *96*, 68–76. https://doi.org/10.1016/j.bios.2017.04.025

Kim, Y. S., Ahmad Raston, N. H., & Gu, M. B. (2016). Aptamer-based nanobiosensors. *Biosensors and Bioelectronics*, *76*, 2–19.

Kokkinos, C., Economou, A., & Prodromidis, M. I. (2016). Electrochemical immunosensors: Critical survey of different architectures and transduction strategies. *Trends in Analytical Chemistry*, *79*, 88–105. https://doi.org/10.1016/j.trac.2015.11.020

Krishnan, S. K., Singh, E., Singh, P., Meyyappan, M., & Nalwa, H. S. (2019). A review on graphene-based nanocomposites for electrochemical and fluorescent biosensors. *RSC Advances*, *9*(16), 8778–8781. https://doi.org/10.1039/c8ra09577a

Krishnan, S., Tadiboyina, R., Chavali, M., Nikolova, M. P., Wu, R.-J., Bian, D., Jeng, Y.-R., Rao, P. T. S. R. K. P., Palanisamy, P., & Pamanji, S. R. (2019). Graphene-Based Polymer Nanocomposites for Sensor Applications. In *Hybrid Nanocomposites* (Issue April). https://doi.org/10.1201/9780429000966-1

Lah, Z. M. A. N. H., Alang Ahmad, S. A., Zaini, M. S., & Kamarudin, M. A. (2019). An electrochemical sandwich immunosensor for the detection of HER2 using antibody-conjugated PbS quantum dot as a label. *Journal of Pharmaceutical and Biomedical Analysis*, *174*, 608–617. https://doi.org/10.1016/j.jpba.2019.06.024

Leng, C., Lai, G., Yan, F., & Ju, H. (2010). Gold nanoparticle as an electrochemical label for inherently crosstalk-free multiplexed immunoassay on a disposable chip. *Analytica Chimica Acta*, *666*(1–2), 97–101. https://doi.org/10.1016/j.aca.2010.03.060

Li, K., Guo, D., Chen, J., Kong, Y., & Xue, H. (2015). Oil-water interfacial synthesis of graphene-polyaniline-MnO2 hybrids using binary oxidant for high performance supercapacitor. *Synthetic Metals*, *209*, 555–560. https://doi.org/10.1016/j.synthmet.2015.09.017

Li, L., Yuan, Y., Chen, Y., Zhang, P., Bai, Y., & Bai, L. (2018). Aptamer based voltammetric biosensor for Mycobacterium tuberculosis antigen ESAT-6 using a nanohybrid material composed of reduced graphene oxide and a metal-organic framework. *Microchimica Acta*, *185*, 379. https://doi.org/10.1007/s00604-018-2884-5

Li, N., Wang, Y., Cao, W., Zhang, Y., Yan, T., Du, B., & Wei, Q. (2015). An ultrasensitive electrochemical immunosensor for CEA using MWCNT-NH2 supported PdPt nanocages as labels for signal amplification. *Journal of Materials Chemistry B*, *3*, 2006–2011. https://doi.org/10.1039/c4tb01695e

Li, Y., Zhang, Y., Jiang, L., Chu, P. K., Dong, Y., & Wei, Q. (2016). A sandwich-type electrochemical immunosensor based on the biotin- streptavidin-biotin structure for detection of human immunoglobulin G. *Scientific Reports*, *6*(February), 1–9. https://doi.org/10.1038/srep22694

Lim, S. A., Yoshikawa, H., Tamiya, E., Yasin, H. M., & Ahmed, M. U. (2014). A highly sensitive gold nanoparticle bioprobe based electrochemical immunosensor using screen printed graphene biochip. *RSC Advances*, *4*, 58460–58466. https://doi.org/10.1039/c4ra11066h

Liu, A., Wang, K., Weng, S., Lei, Y., Lin, L., Chen, W., Lin, X., & Chen, Y. (2012). Development of electrochemical DNA biosensors. *TrAC - Trends in Analytical Chemistry*, *37*, 101–111. https://doi.org/10.1016/j.trac.2012.03.008

Liu, C., Jang, D., Xiang, G., Liu, F., Liu, L., & Pu, X. (2014). An electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis, based on signal amplification of graphene and a gold nanoparticles-polyaniline nanocomposite. *Analyst*, *139*, 5460–5465. https://doi.org/10.1039/C4AN00976B

Mat Zaid, M. H., Abdullah, J., Yusof, N. A., Sulaiman, Y., Wasoh, H., Md Noh, M. F., & Issa, R. (2017). PNA biosensor based on reduced graphene oxide/water soluble quantum dots for the detection of Mycobacterium tuberculosis. *Sensors and Actuators B: Chemical*, *241*, 1024–1034. https://doi.org/10.1016/j.snb.2016.10.045

Ministry of Health Malaysia. (2018). Country Progress Report on HIV / AIDS. In *Country Progress Report on HIV/AIDS*. http://www.moh.gov.my/resources/index/Penerbitan/Laporan/Report_GAM_2018_Final_(2).pdf

Mishra, G. K., Sharma, V., & Mishra, R. K. (2018). Electrochemical aptasensors for food and environmental safeguarding: A review. *Biosensors*, *8*(28), 1–13. https://doi.org/10.3390/bios8020028

Mohamad, F. S., Mat Zaid, M. H., Abdullah, J., Zawawi, R. M., Hong Ngee, L., Sulaiman, Y., & Rahman, N. A. (2017). Synthesis and characterization of polyaniline/graphene composite nanofiber and its application as an electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis. *Sensors*, *17*(2789), 1–17. https://doi.org/10.3390/s17122789

Mohd Bakhori, N., Yusof, N. A., Abdullah, J., Wasoh, H., Ab Rahman, S. K., & Abd Rahman, S. F. (2020). Surface enhanced CdSe/ZnS QD/SiNP electrochemical immunosensor for the detection of Mycobacterium tuberculosis by combination of CFP10-ESAT6 for better diagnostic specificity. *Materials*, *13*(149), 1–15. https://doi.org/https://doi.org/10.3390/ma13010149

Mohd Bakhori, N., Yusof, N. A., Abdullah, J., Wasoh, H., Md Noor, S. S., Ahmad Raston, N. H., & Mohammad, F. (2018). Immuno Nanosensor for the Ultrasensitive Naked Eye Detection of Tuberculosis. *Sensors*, *18*(6), 1932. https://doi.org/10.3390/s18061932

Mukundan, H., Kumar, S., Price, D. N., Ray, S. M., Lee, Y. J., Min, S., Eum, S., Kubicek-Sutherland, J., Resnick, J. M., Grace, W. K., Anderson, A. S., Hwang, S. H., Cho, S. N., Via, L. E., Barry, C., Sakamuri, R., & Swanson, B. I. (2012). Rapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor. *Tuberculosis*, *92*, 407–416. https://doi.org/10.1016/j.tube.2012.05.009

Omar, N. A. S., Fen, Y. W., Abdullah, J., Chik, C. E. N. C. E., & Mahdi, M. A. (2018). Development of an optical sensor based on surface plasmon resonance phenomenon for diagnosis of dengue virus E-protein. *Sensing and Bio-Sensing Research*, *20*, 16–21. https://doi.org/10.1016/j.sbsr.2018.06.001

Parkhey, P., & Mohan, S. V. (2019). Biosensing applications of microbial fuel cell: Approach toward miniaturization. In S. V. Mohan, S. Varjani, & A. Pandey (Eds.), *Biomass, Biofuels, Biochemicals, Microbial Electrochemical Technology* (pp. 977–997). Elsevier. https://doi.org/10.1016/B978-0-444-64052-9.00040-6

Phan, L. M. T., Rafique, R., Baek, S. H., Nguyen, T. P., Park, K. Y., Kim, E. B., Kim, J. G., Park, J. P., Kailasa, S. K., Kim, H.-J., Chung, C., Shim, T. S., & Park, T. J. (2018). Gold-copper nanoshell dot-blot immunoassay for naked-eye sensitive detection of tuberculosis specific CFP-10 antigen. *Biosensors and Bioelectronics*, *121*, 111–117. https://doi.org/https://doi.org/10.1016/j.bios.2018.08.068

Roberts, A., Tripathi, P. P., & Gandhi, S. (2019). Graphene nanosheets as an electric mediator for ultrafast sensing of urokinase plasminogen activator receptor-A biomarker of cancer. *Biosensors and Bioelectronics*, *141*, 111398. https://doi.org/10.1016/j.bios.2019.111398

Rooyanian, S., Bagherzadeh, M., Akrami, Z., & Golikand, A. N. (2018). A simple route to surface functionalization of graphene nanosheets by

benzoic acid and its application toward Pb(ii) sensing. *New Journal of Chemistry*, *42*, 17371–17378. https://doi.org/10.1039/C8NJ04110E

Rotherham, L. S., Maserumule, C., Dheda, K., Theron, J., & Khati, M. (2012). Selection and Application of ssDNA Aptamers to Detect Active TB from Sputum Samples. *PLoS ONE*, *7*(10), e46862. https://doi.org/10.1371/journal.pone.0046862

Sassolas, A., Blum, L. J., & Béatrice, D. L. B. (2009). Electrochemical aptasensors. *Electroanalysis*, *21*(11), 1237–1250. https://doi.org/10.1002/elan.200804554

Senel, M., Dervisevic, M., & Kokkokoğlu, F. (2019). Electrochemical DNA biosensors for label-free breast cancer gene marker detection. *Analytical and Bioanalytical Chemistry*, *411*, 2925–2935. https://doi.org/10.1007/s00216-019-01739-9

Sharma, V., Hynek, D., Trnkova, L., Hemzal, D., Marik, M., Kizek, R., & Hubalek, J. (2016). Electrochemical determination of adenine using a glassy carbon electrode modified with graphene oxide and polyaniline. *Microchimica Acta*, *183*, 1299–1306. https://doi.org/10.1007/s00604-015-1740-0

Shoaie, N., Daneshpour, M., Azimzadeh, M., Mahshid, S., Khoshfetrat, S. M., Jahanpeyma, F., Gholaminejad, A., Omidfar, K., & Foruzandeh, M. (2019). Electrochemical sensors and biosensors based on the use of polyaniline and its nanocomposites: a review on recent advances. *Microchimica Acta, 186*(465), 1–29. https://doi.org/10.1007/s00604-019-3588-1

Silva, S. M., Tavallaie, R., Sandiford, L., Tilley, R., & Gooding, J. J. (2016). Gold Coated Magnetic Nanoparticles: Preparation, Surface Modification for Analytical and Biomedical Applications. *Chem. Commun.*, *52*, 7528–7540. https://doi.org/10.1039/C6CC03225G

Skládal, P. (2016). Piezoelectric biosensors. *Trends in Analytical Chemistry*, *79*, 127–133. https://doi.org/http://dx.doi.org/10.1016/j.trac.2015.12.009

Srivastava, S. K., van Rijn, C. J. M., & Jongsma, M. A. (2016). Biosensor-based detection of tuberculosis. *RSC Advances*, *6*, 17759–17771. https://doi.org/10.1039/C5RA15269K

Sun, J. R., Lee, S. Y., Perng, C. L., & Lu, J. J. (2009). Detecting Mycobacterium tuberculosis in bactec MGIT 960 cultures by inhouse IS6110-based PCR assay in routine clinical practice. *Journal of the Formosan Medical Association*, *108*(2), 119–125. https://doi.org/10.1016/S0929-6646(09)60042-5

Sypabekova, M., Jolly, P., Estrela, P., & Kanayeva, D. (2019). Electrochemical aptasensor using optimized surface chemistry for the detection of Mycobacterium tuberculosis secreted protein MPT64 in human serum. *Biosensors and Bioelectronics*, *123*, 141–151.

https://doi.org/10.1016/j.bios.2018.07.053

Tang, X. L., Zhou, Y. X., Wu, S. M., Pan, Q., Xia, B., & Zhang, X. L. (2014). CFP10 and ESAT6 aptamers as effective Mycobacterial antigen diagnostic reagents. *Journal of Infection*, *69*(6), 569–580. https://doi.org/10.1016/j.jinf.2014.05.015

Teixeira, S., Conlan, R. S., Guy, O. J., & Sales, M. G. F. (2014). Label-free human chorionic gonadotropin detection at picogram levels using oriented antibodies bound to graphene screen-printed electrodes. *Journal of Materials Chemistry B*, *2*(13), 1852–1865. https://doi.org/10.1039/c3tb21235a

Thammasoontaree, N., Rattanarat, P., Ruecha, N., Siangproh, W., Rodthongkum, N., & Chailapakul, O. (2014). Ultra-performance liquid chromatography coupled with graphene/polyaniline nanocomposite modified electrode for the determination of sulfonamide residues. *Talanta*, *123*, 115–121. https://doi.org/10.1016/j.talanta.2014.02.004

Thevenot, D. R., Toth, K., Durst, R. A., & Wilson, G. S. (1999). Electrochemical Biosensors: Recommended Definitions and Classification. *Pure and Applied Chemistry*, *71*(12), 2333–2348.

Torati, S. R., Reddy, V., Yoon, S. S., & Kim, C. (2016). Electrochemical biosensor for Mycobacterium tuberculosis DNA detection based on gold nanotubes array electrode platform. *Biosensors and Bioelectronics*, *78*, 483–488. https://doi.org/10.1016/j.bios.2015.11.098

Tuerk, C., & Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, *249*, 505–510. https://doi.org/10.1126/science.2200121

Tufa, L. T., Oh, S., Tran, V. T., Kim, J., Jeong, K. J., Park, T. J., Kim, H. J., & Lee, J. (2018). Electrochemical immunosensor using nanotriplex of graphene quantum dots, Fe3O4 and Ag nanoparticles for tuberculosis. *Electrochimica Acta*, *290*, 369–377. https://doi.org/10.1016/j.electacta.2018.09.108

Wang, C. F., Sun, X. Y., Su, M., Wang, Y. P., & Lv, Y. K. (2020). Electrochemical biosensors based on antibody, nucleic acid and enzyme functionalized graphene for the detection of disease-related biomolecules. *Analyst*, *145*(5), 1550–1562. https://doi.org/10.1039/c9an02047k

Wang, Li, Lu, X., Lei, S., & Song, Y. (2014). Graphene-based polyaniline nanocomposites: preparation, properties and applications. *Journal of Materials Chemistry A*, *2*(13), 4491–4509. https://doi.org/10.1039/C3TA13462H

Wang, Lisong, Leng, C., Tang, S., Lei, J., & Ju, H. (2012). Enzyme-free signal amplification for electrochemical detection of Mycobacterium lipoarabinomannan antibody on a disposable chip. *Biosensors and Bioelectronics*, *38*, 421–424. https://doi.org/10.1016/j.bios.2012.05.025

Wang, X., Han, X., Ma, A., Chen, L., Liang, H., Litifu, A., & Xue, F. (2017). Fabrication of electrochemical immunosensor for interferon-γ determination and its application of tuberculosis diagnosis. *International Journal of Electrochemical Science*, *12*, 7262–7271. https://doi.org/10.20964/2017.08.67

Wang, Y. (2018). Preparation and application of polyaniline nanofibers: an overview. *Polymer International*, *67*(6), 650–669. https://doi.org/10.1002/pi.5562

Wei, Q., Xiang, Z., He, J., Wang, G., Li, H., Qian, Z., & Yang, M. (2010). Dumbbell-like Au-Fe3O4 nanoparticles as label for the preparation of electrochemical immunosensors. *Biosensors and Bioelectronics*, *26*, 627–631. https://doi.org/10.1016/j.bios.2010.07.012

World Health Organization. (2015). *Global Tuberculosis Report 2015*.

World Health Organization. (2018). *Global Tuberculosis Report 2018*.

World Health Organization. (2019). *Global tuberculosis report 2019*.

World Health Organization. (2020). *Global tuberculosis report 2020*.

Yin, H., Wang, H., Jiang, W., Zhou, Y., & Ai, S. (2017). Electrochemical immunosensor for N6-methyladenosine detection in human cell lines based on biotin-streptavidin system and silver-SiO2 signal amplification. *Biosensors and Bioelectronics*, *90*, 494–500. https://doi.org/10.1016/j.bios.2016.10.066

Yu, H., Chen, M., Rice, P. M., Wang, S. X., White, R. L., & Sun, S. (2005). Dumbbell-like bifunctional Au−Fe3O4 nanoparticles. *Nano Letters*, *5*(2), 379–382. https://doi.org/https://doi/10.1021/nl047955q

Zhang, J., Li, Y., Duan, S., & He, F. (2020). Highly electrically conductive two-dimensional Ti3C2 Mxenes-based 16S rDNA electrochemical sensor for detecting Mycobacterium tuberculosis. *Analytica Chimica Acta*, *1123*, 9–17. https://doi.org/10.1016/j.aca.2020.05.013

Zhang, Y., Zhang, B., Ye, X., Yan, Y., Huang, L., Jiang, Z., Tan, S., & Cai, X. (2016). Electrochemical immunosensor for interferon- γ based on disposable ITO detector and HRP-antibody-conjugated nano gold as signal tag. *Materials Science and Engineering C*, *59*, 577–584. https://doi.org/https://doi.org/10.1016/j.msec.2015.10.066

Zhao, X., Cai, Y., Wang, T., Shi, Y., & Jiang, G. (2008). Preparation of alkanethiolate-functionalized core/shell Fe 3O4@Au nanoparticles and its interaction with several typical target molecules. *Analytical Chemistry*, *80*(23), 9091–9096. https://doi.org/10.1021/ac801581m

Zheng, D., Vashist, S. K., Al-Rubeaan, K., Luong, J. H. T., & Sheu, F. S. (2012). Mediatorless amperometric glucose biosensing using 3-aminopropyltriethoxysilane-functionalized graphene. *Talanta*, *99*, 22–28.

https://doi.org/10.1016/j.talanta.2012.05.014

Zheng, Q., Wu, H., Shen, Z., Gao, W., Yu, Y., Ma, Y., Guang, W., Guo, Q., Yan, R., Wang, J., & Ding, K. (2015). An electrochemical DNA sensor based on polyaniline/graphene: High sensitivity to DNA sequences in a wide range. *Analyst*, *140*(19), 6660–6670. https://doi.org/10.1039/c5an01088h

Zhou, H., Lee, J., Park, T. J., Lee, S. J., Park, J. Y., & Lee, J. (2012). Ultrasensitive DNA monitoring by Au-Fe 3O 4 nanocomplex. *Sensors and Actuators B: Chemical*, *163*, 224–232. https://doi.org/10.1016/j.snb.2012.01.040

Zhou, L., He, X., He, D., Wang, K., & Qin, D. (2011). Biosensing technologies for mycobacterium tuberculosis detection: Status and new developments. *Clinical and Developmental Immunology*, *2011*, 193963. https://doi.org/10.1155/2011/193963

BIODATA OF STUDENT

Umi Zulaikha binti Mohd Azmi was born on 4th August 1993 in Hospital Teluk Intan, Perak. She received her primary education in Sekolah Kebangsaan Khir Johari, Sabak Bernam, Selangor and secondary school in Sekolah Menengah Kebangsaan Agama Simpang Lima, Sungai Besar, Selangor. Then, she was managed to complete her foundation study in engineering at UiTM Puncak Alam with flying colours within one year. After finishing her foundation study, she directly pursued her degree study in Bachelor of Science (Chemistry) at Universiti Teknologi Malaysia (UTM) and completed with Second Class (Upper) with Honours in 2016. Three months later, she was offered as Enumerator for six months to help postgraduate students in their research work. Then, she continued her study in Master of Science (Sensor Technology) at Universiti Putra Malaysia. Less than two years, she managed to publish one paper and encouraged her to convert her study from Master to PhD. She received a Graduate Research Fellowship (GRF) from UPM as a scholarship throughout her postgraduate study. She also was offered as a laboratory demonstrator to assist undergraduates in practical sessions during the three years period.

LIST OF PUBLICATIONS

Publications

Mohd Azmi, U. Z., Yusof, N. A., Kusnin, N., Abdullah, J., Suraiya, S., Ong, P. S., Ahmad Raston, N. H., Abd Rahman, S. F., Mohamad Fathil, M. F. (2018), Sandwich Electrochemical Immunosensor for Early Detection of Tuberculosis Based on Graphene/Polyaniline-Modified Screen-Printed Gold Electrode. Sensors. doi:10.3390/s18113926 – Published.

Mohd Azmi, U. Z., Yusof, N. A., Abdullah, J., Alang Ahmad, S. A., Mohd Faudzi, F. N., Ahmad Raston, N. H., Suraiya, S., Ong, P. S., Krishnan, D., Sahar, N. K. (2021), Portable electrochemical immunosensor for detection of *Mycobacterium tuberculosis* secreted protein CFP10-ESAT6 in clinical sputum samples. Microchimica Acta, doi: 10.1007/s00604-020-04669-x – Published

Conferences

Electrochemical immunosensor for detection of *Mycobacterium tuberculosis*. 2nd International Symposium on Advanced Materials & Nanotechnology on 15th-16th August 2018 at The Everly, Putrajaya, Malaysia as oral presenter.

A simple and portable electrochemical immunosensor for detection of *Mycobacterium tuberculosis*. 6th International Conference on Bio-Sensing Technology on 16th-19th June 2019 at Hotel Renaissance, Kuala Lumpur, Malaysia as poster presenter

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

_____

_____

_____

NAME OF STUDENT : _____

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☑	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____ _____

(Signature of Student) (Signature of Chairman of Supervisory Committee)

New IC No/ Passport No.: Name:

Date : Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]