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ELECTROCHEMICAL AND OPTICAL-BASED IMMUNOSENSOR FOR DETECTION OF *Mycobacterium tuberculosis*

NOREMYLIA MOHD BAKHORI

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By

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

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Chairman : Professor Nor Azah Yusof, PhD
Institute : Institute of Advanced Technology

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is a major obstacle for the global that effect the rate of morbidity and mortality. Many attempts and affords had been taken such as developing the drugs for controlling TB, curing the patients and preventing further transmission of the disease. Conventional diagnosis tool of TB often time-consuming, required loads of samples, less sensitive, impractical and costly for point of care diagnostic. In this research, a novel for diagnosis of TB was developed by an optical and electrochemical immunosensor via antibody-antigen interaction for the detection of TB-protein biomarker CFP10-ESAT6. For optical immunosensor, we studied a naked eye detection for TB by utilizing plasmonic enzyme-linked immunosorbent assay (ELISA) for the detection of protein biomarker, Mycobacterium tuberculosis ESAT-6-like protein esxB (CFP10-ESAT6). Here, the biocatalytic cycle of the intracellular enzymes,catalase links to the formation and successive growth of the gold nanoparticles (AuNPs). The formation of blue and red of AuNPs colored solutions links directly to the absence or presence of the TB analytes in the sample solutions. The immunoassay involves catalase-labeled antibodies which consume hydrogen peroxide for reduction of gold (III) chloride and further produce AuNPs. The fast rate of reaction determines the agglomerated of AuNPs for blue solutions while slow reaction for red solution which from monodispersed AuNPs. This serves as a confirmation for the naked eye detection of TB analytes. The optimum concentration of H_2O_2 and gold ion were 150 µM and 0.25 mM respectively. In the presence of CFP10-ESAT6, blue color produced while in the absence of CFP10-ESAT6, red solution appeared. The detection limit (LOD) of-developed method was 0.01 µg/mL by the naked eye. Additionally, the plasmonic ELISA shows high specificity towards CFP10-ESAT6 protein compared with MPT64 and BSA. Furthermore, our developed technique was successfully tested and confirmed with sputum samples from patients diagnosed with positive and negative TB with good reproducibility. The results show only positive TB sputum samples produced blue color solutions compared with negative sputum samples, non-tuberculosis Runyon Group IV and *Mycobacterium fortuitum chelonae* complex. The results provided enough evidence for the utilization of our technique in the early diagnosis of TB disease.

For electrochemical immunosensor, a modified quantum dot with thioglycolic acid (TGA) (CdSe-ZnS QD) and functionalized silica nanoparticles (SiO_2NPs) as modifiers were prepared to enhance performance of disposable screen printed carbon electrode (SPCE). CdSe-ZnS QD was characterized by using fluorescence spectroscopy and High Resolution Transmission Electron Microscopy (HRTEM) while SiNPs with Transmission Electron Microscopy (TEM) and Fourier Transform Infrared (FTIR). Functionalized SiO_2NPs and CdSe-ZnS QD were dropped cast on the working electrode for preparation of CdSe-ZnS QD/ SiO_2NPs/SPCE. Electrochemical studies using cyclic voltammetry (CV) performed with SiO_2NPs/SPCE and CdSe-ZnS QD/SiO_2NPs/SPCE were found to give a better response through the optimization of numerous analytical parameters. The modified SPCE was characterized using Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive X-ray (EDX) respectively. The CdSe-ZnS QD/SiO_2NPs/SPCE modified electrode showed increment of active surface area with 4.14 folds higher than bare SPCE. Then indirect ELISA immunoassay was performed on the modified electrode for CFP10-ESAT6 detection using differential potential voltammetry (DPV). DPV current response increased in the presence of CFP10-ESAT6 while decreased in the absence of CFP10-ESAT6. Other than that, DPV current was high for CFP10-ESAT6 compared with BSA and MPT64. The detection of CFP10-ESAT6 showed a linear response towards different concentration of CFP10-ESAT6 with $R^2 = 0.9487$ for calibration curve. The detection limit of 3.3×10^{-11} g/mL was achieved for linear range of 20 to 100 ng/mL of CFP10-ESAT6 concentration. The proposed methods showed good selectivity and reproducibility of target analyte with RSD value of 1.45 %.

As summary, the developed optical immunosensor utilized plasmonic ELISA marked as suitable quantitative method for naked eye detection of TB. Besides, the developed electrochemical immunosensor which used the fabricated electrode can be used as qualitative technique for TB.

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ELECKTROKIMIA DAN OPTIKAL BERDASARKAN IMUNOSENSOR UNTUK PENGESANAN *Mycobacterium tuberculosis*

Oleh

NOREMYLIA MOHD BAKHORI

Mei 2019

Pengerusi : Profesor Nor Azah Yusof, PhD
Fakulti : Institut Teknologi Maju

Tuberkulosis (TB) yang disebabkan oleh *Mycobacterium tuberculosis* adalah halangan utama bagi global yang mempengaruhi kadar morbiditi dan kematian. Banyak percubaan dan usaha telah diambil seperti mencipta ubat- ubatan untuk mengawal TB, menyembuhkan pesakit dan mencegah penyebaran penyakit ini. Alat pengesan konvensional TB sering memakan masa, memerlukan sampel yang banyak, kurang sensitif, tidak praktikal dan mahal untuk pengesanan yang mudah. Dalam kajian ini, pengesanan TB yang pertama telah dikembangkan menggunakan imunosensor optik dan elektrokimia melalui interaksi antibodi-antigen untuk pengesanan TB protin iaitu CFP10-ESAT6. Bagi imunosensor optik, kami mencipta pengesanan TB secara mata kasar dengan menggunakan ujian imunosorben berkaitan enzim plasmonik (ELISA) untuk pengesanan TB protin iaitu Mycobacterium tuberculosis ESAT-6-like protein esxB (CFP10-ESAT6). Di sini, kitaran biokatalitik enzim intraselular, katalase dihubungkan kepada pembentukan dan pertumbuhan nanopartikel emas (AuNPs). Pembentukan larutan berwarna biru dan merah AuNPs berhubung secara langsung dengan ketiadaan atau kehadiran protin TB dalam sampel. Immunoassay ini melibatkan antibodi berlabel katalase yang menggunakan hidrogen peroksida untuk menurunkan emas (III) klorida dan seterusnya menghasilkan AuNPs. Kadar tindak balas yang cepat menentukan penggumpalan AuNPs untuk menghasilkan larutan biru sementara tindak balas perlahan untuk menghasilkan larutan merah yang berasal dari AuNPs yang tidak bergumpal. Ini berfungsi sebagai pengesahan untuk pengesanan mata kasar terhadap pengesanan TB. Kepekatan optimum untuk H_2O_2 dan ion emas adalah 150 µM dan 0.25 mM. Dalam kehadiran of CFP10-ESAT6, warna biru dihasilkan manakala dalam ketiadaan CFP10-ESAT6, larutan merah terhasil. Had pengesanan kaedah yang dibangunkan adalah 0.01 µg/mL oleh mata kasar. Tambahan pula, plasmonik ELISA menunjukkan spesifisiti tinggi terhadap protein CFP10-ESAT6 berbanding MPT64 dan BSA. Selain itu, teknik yang dicipta oleh kami telah berjaya diuji dan disahkan dengan sampel kahak dari pesakit yang didiagnosis dengan TB positif dan negatif dengan kebolehulangan yang bagus. Hasilnya

menunjukkan hanya sampel kahak positif menghasilkan larutan warna biru dibandingkan dengan sampel kahak negative, non-tuberculosis Runyon Group IV and *Mycobacterium fortuitum chelonae* kompleks. Keputusan ini memberikan bukti yang mencukupi untuk penggunaan teknik ini dalampengesanan awal penyakit TB.

Bagi imunosensor elektrokimia, kuantum dot yang diubahsuai dengan asid thioglycolic (TGA) (CdSe/ZnS QD) dan nanopartikel silika (SiO_2NPs) sebagai pengubah telah disediakan untuk meningkatkan prestasi elektrod karbon skrin bercetak (SPCE). CdSe-ZnS QD telah di cirikan oleh spektroskopi fluorescence dan High Resolution Transmission Electron Microscopy (HRTEM) manakala SiO_2NPs dengan Transmission Electron Microscopy (TEM) dan Fourier Transform Infrared (FTIR). SiO_2NPs dan CdSe-ZnS QD di letakkan di atas elektrod kerja untuk menghasilkan CdSe-ZnS QD/SiO_2NPs/SPCE. Kajian elektrokimia menggunakan cyclic voltammetry (CV) dijalankan dengan SiO_2NPs/SPCE and CdSe-ZnS QD/SiO_2NPs/SPCE menunjukkan tindak balas yang lebih baik melalui pengoptimuman parameter analitikal. SPCE yang diubahsuai di characterized menggunakan Field Emission Scanning Electron Microscope (FESEM) dan Energy Dispersive X-ray (EDX). Elektrod yang diubahsuai CdSe-ZnS QD/SiO_2NPs/SPCE menunjukkan pertambahan luas permukaan aktif dengan 4.14 lebih tinggi daripada SPCE yang tidak diubahsuai. Kemudian, ELISA imunosorbent tidak terus dijalankan oleh elektrod yang diubahsuai untuk pengesanan CFP10-ESAT6 menggunakan potential voltammetry (DPV). Tindak balas arus DPV bertambah dalam kehadiran CFP10-ESAT6 manakala berkurangan dalam ketiadaan CFP10-ESAT6. Selain daripada itu, arus DPV adalah tinggi untuk CFP10-ESAT6 berbanding dengan BSA dan MPT64. Pengesanan CFP10-ESAT6 menunjukkan tindak balas linear ke arah kepekatan yang berbeza oleh CFP10-ESAT6 dengan $R^2 = 0.9487$ untuk keluk penentukuran. Had pengesanan ialah 3.3×10^{-11} g/mL dicapai untuk julat 20 hingga 100 ng/mL bagi kepekatan CFP10-ESAT6. Kaedah yang dicadangkan menunjukkan pemilihan dan kebolehulangan yang baik untuk lima ukuran di mana nilai RSD adalah sebanyak 1.45%.

Sebagai ringkasan, satu imunosensor optik telah dikembangkan menggunakan plasmonik ELISA telah ditanda sebagai satu kaedah kuantitatif yang sesuai untuk pengesanan TB secara mata kasar. Selain itu, imunosensor elekrokimia telah dikembangkan yangmana menggunakan elektrod yang telah difabrikasikan.untuk pengesanan TB secara kualitatif

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

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

Helmi Wasoh, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecule Science
Universiti Putra Malaysia
(Member)

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Signature:
Name of Member of Supervisory Committee: Dr. Jaafar Bin Abdullah

Signature:
Name of Member of Supervisory Committee: Dr. Helmi Bin Wasoh

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LIST OF ABBREVIATIONS

AFB	Acid-Fast Bacilli
AP	Alkaline Phosphatase
APTES	Aminopropyltriethoxysilane
AuNPs	Gold Nanoparticles
BCG	Bacillus Calmette-Guerin
CNT	Carbon Nanotubes
CSF	Cerebrospinal Fluid
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EDX	Energy Dispersive X-Ray
ELISA	Enzyme-Linked Immunosorbent Assay
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
HRP	Horseradish Peroxide
HRTEM	High Resolution Transmission Electron Microscopy
IGRA	Interferon-Gamma Release Assay
LED	Light-Emitting Diode
LOD	Limit of Detection
LPA	Line-Probe Assays
LSPR	Localized Surface Plasmon Resonance
LSV	Linear Sweep Voltammetry
MGIT	Mycobacteria Growth Indicator Tube
NAAT	Nucleic Acid Amplications Tests
PCR	Polymerase Chain Reaction
PSA	Prostate-Specific Antigen
QD	Quantum Dot
SiNPs	Silica Nanoparticles
SiNW-FET	Silicon Nanowire Field-Effect Transistors
SPCE	Screen Printed Carbon Electrode
SPE	Screen-Printed Electrodes
SPR	Surface Plasmon Resonance
ssDNA	Single Strand Dna
SWV	Square Wave Voltammetry
TEM	Transmission Electron Microscopy
UV-Vis	Ultraviolet-Visible
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Tuberculosis (TB) is the leading cause of death from bacterial infectious disease (McNerney et al., 2012). In 2016, about 10.4 million people were affected by TB around the world which there were 1.7 million cases contributed for TB-death worldwide including people who are TB-HIV infected (WHO, 2017). In Malaysia, it has become the major public health issue (William et al., 2015) . According to the Health Ministry of Malaysia, the TB cases jumped by 6 % to 25,739 in 2016 compared with 24,220 in 2015 which the total number of death increased 15 % from 1,696 to 1,945 (Bernama, 2017). Foreign worker became the main contributor for spreading the TB disease with the death rate higher than dengue cases in this country due to the high rate of human migration from other TB burden countries such as Indonesia, Vietnam, Cambodia, Philippines and Vietnam (Ng, 2014).

TB caused by an airbone bacterial infection from *Mycobacterium tuberculosis* (Mtb) (Dande & Samant, 2018) Usually, TB attacked the lungs (pulmonary TB) and another part of the body such as kidney, brain and spine. TB disease in the lungs and throats are infectious because the bacteria can spread to other people through the air. People that experienced active TB exhibit symptoms like bad cough and pain in chest. The main aim in treatment of TB is to cure the patients and to prevent the spread of TB and the development of drug resistant TB (Kannabus, 2017).

In recent years, there are several tests that is available for diagnostic of Mtb including microscopy (Ahmad et al., 2016; Kidenya et al., 2017), serological test (Reddy et al., 2002; Suraiya et al., 2012), nucleic acid amplication test (NAAT) (Yamamoto et al., 2017; Tammam et al., 2017; Bizid et al., 2017) and interferon gamma release assay combined tuberculin skin test (Tama et al., 2017; Kruczak et al., 2016). However, most of these techniques have low sensitivity and specificity, time-consuming, inconsistent, involve multiple specimen per patients, highly-cost and high-burden setting. Furthermore, the extensive spreading of Mtb especially in pulmonary, drug-resistance and HIV-infected TB currently lack accurate TB test.

The utilization antigen-antibody complex in immunonanosensor can improve these drawbacks of the current strategies to increase the performance of diagnostic tools for TB. Generally, immunonanosensors are compact analytical devices in which the event of formation of antigen-antibody complexes is detected and converted, by means of transducer, to an electrical signal which the output can be processed, recorded and displayed (Moina and Ybarra, 2012). The types of transducer are classified according to the signal generation such as electrochemical transducer (Güner et al., 2017; Mouli et al., 2017), optical transducer (Liu et al., 2016; Bhardwaj et al., 2017) and piezoelectric transducer (Su & Li, 2004; Hong et al., 2009). However, optical transducers are most widely used due to advantages of applying visible radiation, nondestructive operation mode, rapid signal generation and highly versatility for clinical application (Luppa et al., 2001).

In optical immunosensor, the major purpose is to detect the presence of antigen or antibodies in body fluids which bind between antigen-antibody complex. It is highly stable to be immobilized on the transducer, then either an optical signal is generated (eg. color or fluorescence) or a change in the optical properties of the surroundings following the antigen-antibody complex formation is measured (Moina and Ybarra 2012). Furthermore, the utilizing of optical immunosensor with enzyme-linked immunosorbent assay (ELISA) is a promising way for naked eye detection purpose with cost-effective, simple, rapid and highly specific detection. However, this assay only generated qualitative results (yes or no) as to whether an antigen is present or not. Hence, the electrochemical immunosensor was employed for obtaining the quantitative results to support the optical immunosensor finding. Electrochemical immunosensor employs the antibody as the capture agent and quantitatively measure the electrical signal resulting from the binding event between the antibody and the target molecule or antigen which the signal often comes from catalytic reaction of enzyme molecule labeled as a signal tracer with detection antibody (Cho et al., 2018). The products containing electric charges can be detected by electrode, thereby enable a sensor device measurement for point-of-care (POC) testing (Kaushik et al., 2018).

In order to access the presence of the proteins (antigen or antibody), immunoassay called marked as the gold standard clinical diagnostic tool which produces signal in form of colors. ELISA commonly contributed in determination the presence of pathogens such as bacteria (Cho & Irudayaraj, 2013; Mirhosseini et al., 2017) and viruses (Alcon et al., 2002; Yu et al., 2017) in food quality control, environmental pollutants and disease biomarkers.

Briefly, ELISA is a rapid plate-based assay for detection and/or quantifying a target antigen or antibodies in a heterogeneous mixture by utilizing enzyme-linked antibodies and chromogenic measurement. The enzyme conjugated antibodies, typically alkaline phosphatase (AP) or horseradish peroxide (HRP) acts as amplifier of detection signal by converting a substrate that results in color change and read by ELISA plate reader (Crowther, 1995). There are several types of ELISA that is depend on which components to be measured such as direct (Nouri et al., 2017) indirect (Nieto et al., 2015) , sandwich (Engvall, 2010) and competitive ELISA (Dea et al., 2000). In addition, this assay is known to be rapid, simple, highly specific and sensitive, and easily automated (Aydin, 2015).

1.2 Problem Statement

Tuberculosis is the major infectious disease that lacks accurate of rapid POC diagnostic tests. Most of the cases of TB reported at the resource strained countries have high burden of disease and death from TB. Currently, direct sputum smear microscopy is the primarily clinical diagnostic tool for tuberculosis in low and middle income countries that employed fast, inexpensive and specific for Mtb (Deka et al., 2016). However, the problem arises from this technique which has low sensitivity that is grossly compromised when the bacterial load is less than 10,000 organisms/mL sputum sample, the patients required serial sputum examination, involves big number of samples (Steingart et al., 2006) and required about 2 weeks to confirm whether the samples taken from the patients contained the bacteria or not.

In order to overcome the limitation from the technique mentioned earlier, ELISA assay is highly suggested instead of using direct sputum smear microscopy because any target molecules can potentially be detected as long as the antibodies against it are available. Conventional ELISA assay promised naked eye detection in large variation concentration of analytes. Besides, the intensively colored solutions were generated in the microtiter plate in the presence of high concentration of analytes. Thus, it is possible to detect the presence of desired analyte with the naked eye when high concentration of analytes is present using conventional ELISA. In the absence and ultralow of analytes, noncolored and lightly colored solutions were generated respectively which can be confusing to differentiate. Apart from this in real samples, even in the absence of target molecules, nonspecific interaction between elements can contribute to the generation of lightly colored solution. Often, the current strategies for ultrasensitive detection of TB required expensive and sophisticated instrumentation.

In our approach, we adapted plasmonic ELISA as optical immunosensor utilizing gold nanoparticles (AuNPs) to overcome the limitation of conventional ELISA for detection naked eye of TB at ultralow concentration with high confident level results by easily distinguishable colors of AuNPs of blue and red colors compared with conventional ELISA only noncolored and lightly colored solutions were generated at absence and ultralow of analytes respectively. Thus, the suitability of the hydrogen peroxide (H_2O_2) as enzyme catalase substrate to yield blue and red colors for naked eye detection at ultralow of analyte can improve the immunosensor for detection of TB. The high sensitivity of H_2O_2 towards immunoreaction provided good naked eye detection. Presently, the utilization of this plasmonic ELISA for detection of TB is not yet reported. Hence, this approach can contribute a new standard diagnosis for TB detection in clinical purpose. This plasmonic ELISA offers advantages such as not required expensive instrumentation, cost-effective, quick response time than gold standard method and suitable for detection in resource-constrain countries. Additionally, the sensor principle of this study was also supported by constructed electrochemical immunosensor for detection of desired protein, CFP10-ESAT6.

In addition, electrochemical methods with the utilizing of screen-printed carbon electrode (SPCE) offers various advantages such as inexpensive, portable, and simple to operate. Nanomaterials such as silica nanoparticles (SiNPs) and CdSe-ZnS quantum dot (CdSe-ZnS QD) can improve the sensing devices due to their unique chemical, physical and electronic properties. In this study, the fabrication of SiNPs/SPCE and CdSe-ZnS QD/SiNPs/SPCE modified electrode were presented as a new strategy to improve the electrochemical immunosensor for detection of CFP10-ESAT6 protein using differential pulse voltammetry (DPV) technique. The utilizing of SiNPs with CdSe-ZnS QD on electrode surface can improve the sensing device and show a good electrocatalytic performance. To date, the utilization combination of SiNPs and CdSe-ZnS QD as modifier in the electrochemical sensor for CFP10-ESAT6 detection has not been reported. The electrochemical method based on SiNPs and CdSe-ZnS QD was used in this research as it offers high sensitivity and selectivity, low cost, portability and short analytical time measurement of CFP10-ESAT6 for POC requirement in diagnostic of TB.

1.3 Objectives

The aim of the study is to develop sensitive detection system based on plasmonic ELISA technique and electrochemical immunosensor based on SiO_2NPs/SPCE and CdSe-ZnS QD/SiO_2NPs/SPCE for tuberculosis detection. The specific objectives are listed below:

1) To investigate the immunoreaction between antigen (CFP10-ESAT6) and antibody (anti CFP10-ESAT6) using plasmonic ELISA.
2) To optimize the sensing capability of the immunosensor utilized the developed plasmonic ELISA.
3) To employ the developed sensing system with real sample (sputum).
4) To fabricated CdSe-ZnS QD/SiO_2NPs/SPCE modified electrode for electrochemical detection of CFP10-ESAT6.
5) To evaluate the analytical performance of the developed sensor of CdSe-ZnS QD/SiNPs/SPCE for electrochemical detection of CFP10-ESAT6 using differential pulse voltammetry (DPV).

1.4 Scope and Limitation

In this study, the attachment of primary antibodies (mouse monoclonal anti CFP-ESAT6) are specific to the CFP10-ESAT6 protein. Therefore, there is limitation in this study when employing optical and electrochemical immunosensor for the other types of protein and antibodies for detection of tuberculosis. The antigen very selective toward its antibody for binding in the immunoassay. The antigen binding site on antibody that is called paratope only recognize its specific antigen to bind on it. Thus, the binding with another types of antigen towards antibody caused no binding between the antigen and antibody. Hence, no biocatalytic enzyme with the substrate for signal generation. Furthermore, there is also limited access of the sputum real samples in application for developed electrochemical immunosensor. This is because of the etiquette from HUSM laboratory that need to be followed before we could access for the sputum samples. This protocol needs time and involved complicated procedures.

REFERENCES

Abramo, C., Meijgaarden, K. E., Garcia, D., Franken, K. L. M. C., Klein, M. R., Kolk, A. J., and Teixeira, H. C. (2006). Monokine induced by interferon gamma and IFN-γ response to a fusion protein of Mycobacterium tuberculosis ESAT-6 and CFP-10 in Brazilian tuberculosis patients. *Microbes and Infection*, *8*(1), 45–51.

Achterberg, E. P., Gledhill, M., Hawkes, J. A., & Avendano, L. C. (2013). Voltammetry-Cathodic Stripping. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, (May), 1–10.

Adha, M. S., Zunaina, E., Liza-Sharmini, A. T., Wan-Hazabbah, W. H., Shatriah, I., Mohtar, I., & Adil, H. (2017). Ocular tuberculosis in Hospital Universiti Sains Malaysia – A case series. *Annals of Medicine and Surgery*, *24*(July), 25–30.

Adhi, B., Alom, A., & Khari, M. (2016). Graphene-based portable SPR sensor for the detection of Mycobacterium tuberculosis DNA strain. *Procedia Engineering*, *168*, 541–545.

Ahmad, T., Ayub, M., Nasir, M., & Khattak, K. (2016). Prevalence of sputum smear positive pulmonary tuberculosis at Dargai , District Malakand , Pakistan : A four year retrospective study. *Egyptian Journal of Chest Diseases and Tuberculosis*, *65*(2), 461–464.

Al-Darraji, H. A. A., Wong, K. C., Yeow, D. G. E., Fu, J. J., Loeliger, K., Paiji, C. & Altice, F. L. (2014). Tuberculosis screening in a novel substance abuse treatment center in Malaysia: Implications for a comprehensive approach for integrated care. *Journal of Substance Abuse Treatment*, *46*(2), 144–149.

Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V., & Flamand, M. (2002). Enzyme-Linked Immunosorbent Assay Specific to Dengue Virus Type 1 Nonstructural Protein NS1 Reveals Circulation of the Antigen in the Blood during the Acute Phase of Disease in Patients Experiencing Primary or Secondary Infections. *Journal of Clinical Microbiology*, *40*(2), 376–381.

Alfonso-Prieto, M., Biarnés, X., Vidossich, P., & Rovira, C. (2009). The Molecular Mechanism of the Catalase Reaction. *Journal of the American Chemical Society*, *131*(33), 11751–11761.

Alvarez-toral, A., Fernández, B., Malherbe, J., Claverie, F., Pecheyran, C., & Pereiro, R. (2017). Spectrochimica Acta Part B Synthesis of amino-functionalized silica nanoparticles for preparation of new laboratory standards. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *138*, 1–7.

Amelia, M., Lincheneau, C., & Credi, A. (2012). Chem Soc Rev Electrochemical properties of CdSe and CdTe quantum dots. *Chem. Soc. Rev.*, *41*, 5728–5743.

Amendola, V., Pilot, R., Frasconi, M., Maragò, O. M., & Iati, M. A. (2017). Surface plasmon resonance in gold nanoparticles: a review. *Journal of Physics: Condensed Matter*, *29*(20), 1–48.

Andreas, J., Hans-Joachim, G., & Claudia, S. (2000). Piezoelectric Mass-Sensing Devices as Biosensors—An Alternative to Optical Biosensors? *Angewandte Chemie International Edition*, *39*(22), 4004–4032.

Arora, J., Kumar, G., Verma, A. K., Bhalla, M., Sarin, R., & Myneedu, V. P. (2015). Utility of MPT64 Antigen Detection for Rapid Confirmation of Mycobacterium tuberculosis Complex. *Journal of Global Infectious Diseases*, *7*(2), 66–69.

Aydin, S. (2015). Peptides A short history , principles , and types of ELISA , and our laboratory experience with peptide / protein analyses using ELISA. *Peptides*, *72*, 4–15.

Azam, S., Rahman, R. T., Lou, Z., & Tang, Y. (2014). Advancements and application of immunosensors in the analysis of food contaminants. *Nusantara Bioscience*, *6*(2), 186–195.

Azmi, M. U. Z., Yusof, N. A., Kusnin, N., Abdullah, J., Suraiya, S., Ong, P. S., Mohamad Fathil, M. F. (2018). Sandwich Electrochemical Immunosensor for Early Detection of Tuberculosis Based on Graphene/Polyaniline-Modified Screen-Printed Gold Electrode. *Sensors*, *18*(11), 3926.

Babu, V. R. S., Patra, S., Karanth, N. G., Kumar, M. A., & Thakur, M. S. (2007). Development of a biosensor for caffeine. *Analytica Chimica Acta*, *582*(2), 329–334.

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, H., Noor, S. S. M., Raston, N. H. A. & Faruq, M. (2018). Immuno Nanosensor for the Ultrasensitive Naked Eye Detection of Tuberculosis. *Sensors*, 18(6), 1932

Balabanova, Y., Drobniewski, F., Nikolayevskyy, V., Kruuner, A., Malomanova, N., Simak, T. & Fedorin, I. (2009). An integrated approach to rapid diagnosis of tuberculosis and multidrug resistance using liquid culture and molecular methods in Russia. *PLoS ONE*, *4*(9), 1–9.

Barnett, R. (2017). Perspectives Case histories Tuberculosis. *The Lancet*, *390*(10092), 351.

Beer, D. De, & Kühl, M. (2001). Interfacial microbial mats and biofilms. *The Benthic Boundary Layer*, *70*(11), 374–394.

Beganskiene, A., Sirutkaitis, V., Kurtinaitiene, M., Juskenas, R. & Kareiva, A. (2004). FTIR, TEM and NMR Iinvestigations of Stöber Silica Nanoparticles. *Material Science*. 10(4) 287-290.

Belluzo, M. S., Ribone, M. É., & Lagier, C. M. (2008). Assembling Amperometric Biosensors for Clinical Diagnostics. *Sensors*, *8*(3), 1366–1399.

Benvidi, A., Rajabzadeh, N., Zahedi, H. M., Mazloum-ardakani, M., Heidari, M. M., & Hosseinzadeh, L. (2015). Simple and Label-free detection of DNA hybridization on a modified graphene nanosheets electrode. *Talanta*, *15*, 1–25.

Bernama. (2017). TB cases increased by 6. Retrieved from http://www.freemalaysiatoday.com/category/nation/2017/03/31/tb-cases-increased-by-6-in-2016-says-minister/

Bhardwaj, N., Bhardwaj, S. K., Nayak, M. K., Mehta, J., & Deep, A. (2017). Fluorescent Nanobiosensors for the Targeted Detection of Foodborne Bacteria. *Trends in Analytical Chemistry*, 61, 7(4).

Bishai, W. (1998). The Mycobacterium tuberculosis genomic sequence: anatomy of a master adaptor. *Trends in Microbiology*, *6*(12), 464–465.

Bizid, S., Blili, S., Mlika, R., Haj Said, A., & Korri-Youssoufi, H. (2017). Direct Electrochemical DNA Sensor based on a new redox oligomer modified with ferrocene and carboxylic acid: Application to the detection of Mycobacterium tuberculosis mutant strain. *Analytica Chimica Acta*, *994*, 10–18.

Bluestein, D. (1988). Monoclonal antibody pregnancy tests. *American Family Physician*, *38*(1), 197–204.

Blumberg, M. H. & Kempker, R. R. (2015). Interferon-γ Release Assays for the Evaluation of Tuberculosis Infection. *Journal of American Medical Association*, *312*(14), 1460–1461.

Bontidean, I., Mortari, A., Leth, S., Brown, N. L., Karlson, U., Larsen, M. M. & Csöregi, E. (2004). Biosensors for detection of mercury in contaminated soils. *Environmental Pollution*, *131*(2), 255–262.

Braet, W. Y., Thordarson, P., Gooding, J. J. & Filip, S. P. R. (2007). Carbon nanotubes for biological and biomedical applications. *Nanotechnology*, *18*(41), 412001.

Brown, L. (1941). The story of clinical pulmonary tuberculosis. In M. H. Brown (Ed.) (2nd ed., pp. 1871–1937). University of Michigan, USA: Baltimore, Md. : The Williams & Wilkins Company.

Bu, A. (2005). Chapter 9. *Fuel*, (3), 119–124.

Cambau, E., & Drancourt, M. (2014). Steps towards the discovery of Mycobacterium tuberculosis by Robert. *Clinical Microbiology and Infection*, *20*(3), 196–201.

Caminero, J. A., Cayla, J. A., García-garcía, J., García-pérez, F. J., Palacios, J. J. & Ruiz-manzano, J. (2017). Diagnosis and Treatment of Drug-Resistant Tuberculosis ☆. *Archivos de Bronconeumología (English Edition)*, *53*(9), 501–509.

Campanella, L., Cubadda, F., Sammartino, M. & Saoncella, A. (2001). An algal biosensor for the monitoring of water toxicity in estuarine environments. *Water Research*, *35*(1), 69–76.

Campbell, I. A., & Bah-sow, O. (2006). Pulmonary tuberculosis : diagnosis and treatment. *BMJ*, *332*(May), 1194–1197.

Castillo, D. S., & Cassola, A. (2017). Novel sensitive monoclonal antibody based competitive enzyme-linked immunosorbent assay for the detection of raw and processed bovine beta-casein. *PLoS ONE*, *12*(7), 1–19.

Cecchin, D., de la Rica, R., Bain, R. E. S., Finnis, M. W., Stevens, M. M., & Battaglia, G. (2014). Plasmonic ELISA for the detection of gp120 at ultralow concentrations with the naked eye. *Nanoscale*, *6*(16), 9559–9562.

Center of Disease Control. (2013). *A New Tool to Diagnose Tuberculosis : The Xpert MTB / RIF Assay*.

Chah, S. (2005). Gold Nanoparticles as a Colorimetric Sensor for Protein Conformational Changes, *12*, 323–328.

Chang, L., Li, J., & Wang, L. (2016). Immuno-PCR: An ultrasensitive immunoassay for biomolecular detection. *Analytica Chimica Acta*, *910*, 12–24.

Chaves, A. S., Rodrigues, M. F., Márcia, A., Mattos, M., & Teixeira, H. C. (2015). Challenging Mycobacterium tuberculosi s dormancy mechanisms and their immunodiagnostic potential. *Brazilian Journal of Infectious Diseases*, *19*(6), 636–642.

Chen, H., Jiang, C., Yu, C., Zhang, S., Liu, B., & Kong, J. (2009). Protein chips and nanomaterials for application in tumor marker immunoassays. *Biosensors and Bioelectronics*, *24*(12), 3399–3411.

Chen, K.-I., Li, B.-R., & Chen, Y.-T. (2011). Silicon nanowire field-effect transistor-based biosensors for biomedical diagnosis and cellular recording investigation. *Nano Today*, *6*(2), 131–154.

Chen, R., Huang, X., Xu, H., Xiong, Y., & Li, Y. (2015). Plasmonic Enzyme-Linked Immunosorbent Assay Using Nanospherical Brushes as a Catalase Container for Colorimetric Detection of Ultralow Concentrations of Listeria monocytogenes. *ACS Applied Materials & Interfaces*, *7*(51), 28632–28639.

Cho, I. H., & Irudayaraj, J. (2013). In-situ immuno-gold nanoparticle network ELISA biosensors for pathogen detection. *International Journal of Food Microbiology*, *164*(1), 70–75.

Cho, I., Lee, J., Kim, J., Kang, M., Paik, J. K., Ku, S., & Kim, D. (2018). Current Technologies of Electrochemical Immunosensors : Perspective on Signal Amplification, 1–18.

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.

Crowther, J. R. (1995). *Elisa*. *Methods in Molecular Biology (Clifton, N.J.)* (Vol. 42).

D. J. Deka, B. Choudhury, P. Talukdar, T. Q. Lo, B. Das, S. A. Nair, P. K., & Moonan, A. M. V. K. (2016). What a difference a day makes: same-day vs. 2-day sputum smear microscopy for diagnosing tuberculosis. *International Union Against Tuberculosis and Lung Disease*, *6*(4), 232–236.

Daftary, V. G., Banker, D. D., & Daftary, G. V. (1994). ELISA test for tuberculosis. *Indian Journal of Medical Sciences*, *48*(2), 39–42.

Damborsky, P., vitel, J., & Katrlik, J. (2016). Optical biosensors. *Essays In Biochemistry*, *60*(1), 91–100.

Kanayeva, D., & Bekniyavoz, I. Z. A. (2013). Detection of Tuberculosis Using Biosensors: Recent Progress and Future Trends. *Sensors & Transducers*, *149*(2), 166–173.

Dande, P., & Samant, P. (2018). Acquaintance to Artificial Neural Networks and use of artificial intelligence as a diagnostic tool for tuberculosis : A review. *Tuberculosis*, *108*, 1–9.

Daniel, T. (1998). The early history of tuberculosis in central East Africa: insights from the clinical records of the first twenty years of Mengo Hospital and review of the relevant literature. *Int J Tuberc Lung Dis*, *2*, 1–7.

de la Rica, R., & Stevens, M. M. (2012). Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. *Nature Nanotechnology*, *7*, 821.

de la Rica, R., & Stevens, M. M. (2013). Plasmonic ELISA for the detection of analytes at ultralow concentrations with the naked eye. *Nature Protocols*, *8*(9), 1759–1764.

Dea, S., Wilson, L., Therrien, D., & Cornaglia, E. (2000). Competitive ELISA for detection of antibodies to porcine reproductive and respiratory syndrome virus using recombinant E. coli-expressed nucleocapsid protein as antigen. *J Virol Methods*, *87*(1–2), 109–122.

Defrances, M. C., & Morgan, W. S. (2006). C-Terminal Signal Sequence, *313*(September), 1632–1637.

Demir, B., Yilmaz, T., Guler, E., Gumus, Z. P., Akbulut, H., Aldemir, E., & Yagci, Y. (2016). Polypeptide with electroactive endgroups as sensing platform for the abused drug ‘methamphetamine’ by bioelectrochemical method. *Talanta*, *161*, 789–796.

Denton, K. A., Kramer, M. F., & Lim, D. V. (2008). Rapid Detection Of

Mycobacterium tuberculosis in luung tissue using a fiber optic biosensor. *Journal of Rapid Methods & Automation in Microbiology*, *17*(2009), 17–31.

Desikan, P., Panwalkar, N., Mirza, S. B., Chaturvedi, A., Ansari, K., Varathe, R., & Pandey, M. (2017). Line probe assay for detection of Mycobacterium tuberculosis complex: An experience from Central India.

Dheda, K., Davids, V., Lenders, L., Roberts, T., Meldau, R., Ling, D., & Zumla, A. (2010). Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. *PloS One*, *5*(3), e9848.

Dinçkaya, E., Akyilmaz, E., Kemal Sezgintürk, M., & Nil Ertaş, F. (2010). Sensitive nitrate determination in water and meat samples by amperometric biosensor. *Preparative Biochemistry and Biotechnology*, *40*(2), 119–128.

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.

Dom, O., & Mart, M. J. A. (2007). Recent developments in the field of screen-printed electrodes and their related applications. *Talanta*, *73*, 202–219. https://doi.org/10.1016/j.talanta.2007.03.050

Drobniewski, F. A., Caws, M., Gibson, A., & Young, D. (2003). Modern laboratory diagnosis of tuberculosis. *Lancet Infect Dis*, *3*(3), 141–147.

Drobniewski, F., Nikolayevskyy, V., Balabanova, Y., Bang, D., & Papaventsis, D. (2012). Diagnosis of tuberculosis and drug resistance: what can new tools bring us? *The International Journal of Tuberculosis and Lung Disease*, *16*(7), 860–870.

Dunn, J. J., Starke, J. R., & Revell, P. . (2016). Laboratory Diagnosis of Mycobacterium tuberculosis Infection and Disease in Children. *Journal of Clinical Microbiology*, *54*(6), 1434–1441.

Elmi, O. S., Ph, D., Zuki, M., Mat, B., Med, M., Abdullah, S. B., & Zilafil, B. A. (2016). Extensively Drug-Resistant Mycobacterium tuberculosis in Kelantan East Coast of Malaysia : First Two Cases. *Clinical Microbiology Newsletter*, *38*(5), 40–42.

Engvall, E. (2010). The ELISA, enzyme-linked immunosorbent assay. *Clinical Chemistry*, *56*(2), 319–320.

Ensafi, A. A., Taei, M., & Khayamian, T. (2009). A differential pulse voltammetric method for simultaneous determination of ascorbic acid , dopamine , and uric acid using poly (3- (5-chloro-2- modified glassy carbon electrode. *Journal of Electroanalytical Chemistry*, *633*(1), 212–220.

Ernst, J. D. (1998). Macrophage Receptors for Mycobacterium tuberculosis

MINIREVIEW Macrophage Receptors for Mycobacterium tuberculosis. *Infection and Immunity*, *66*(4), 1277–1281.

Ernst, J. D. (2012). The immunological life cycle of tuberculosis. *Nature Reviews Immunology*, *12*, 581–591.

Eshetu, G. (2015). *Detection and Characterization of Mycobacterium tuberculosis in Stool of HIV Sero-positive Patients with Suspected Pulmonary Tuberculosis By : Gizaw Eshetu OF HEALTH OF MICROBIOLOGY , IMMUNOLOGY AND PARASITOLOG*. Addis Ababa University.

Esposito, S., Tagliabue, C., & Bosis, S. (2013). Tuberculosis in Children. *Mediterranean Journal of Hematology and Infectious Diseases*, *5*(1).

Faeste, C. K., & Plassen, C. (2008). Quantitative sandwich ELISA for the determination of fish in foods. *Journal of Immunological Methods*, *329*(1–2), 45–55.

Felix, F. S., & Angnes, L. (2017). Author ' s Accepted Manuscript IMMUNOSENSORS – A POWERFUL TOOL FOR ANALYTICAL. *Biosensors and Bioelectronic*.

Feng, T. T., Shou, C. M., Shen, L., Qian, Y., Wu, Z. G., Fan, J., & Yao, H. (2011). Novel monoclonal antibodies to ESAT-6 and CFP-10 antigens for ELISA-based diagnosis of pleural tuberculosis. *International Journal of Tuberculosis and Lung Disease*, *15*(6), 804–810.

Fernandes, K. V., & Machado, O. L. T. (2012). World ' s largest Science , Technology & Medicine Open Access book publisher Approaches for the Detection of Toxic Compounds in Castor and Physic Nut Seeds and Cakes. In Z. Fang (Ed.), *Approaches for the Detection of Toxic Compounds in Castor and Physic Nut Seeds and Cakes,Biodiesel - Feedstocks, Production and Applications Several* (pp. 284–307). InTech.

Flaherty, D. K. (Ed.). (2012). Chapter 14 - Antibodies and In Vitro Research and Diagnostic Assays. In *Immunology for Pharmacy* (pp. 110–117). Saint Louis: Mosby.

Fogel, N. (2015). Tuberculosis : A disease without boundaries. *Tuberculosis*, *95*(5), 527–531.

Forthal, D. N. (2014, August 15). Functions of Antibodies. *Microbiology Spectrum*.

Fracchiolla, N. S., Artuso, S., & Cortelezzi, A. (2013). Biosensors in clinical practice: Focus on oncohematology. *Sensors*, *13*(5), 6423–6447.

Frenzel, A., Hust, M., & Schirrmann, T. (2013). Expression of Recombinant Antibodies. *Frontiers in Immunology*, *4*(217).

Gao, B. L., Liu, J., Dong, L. X., Zhang, L., Qin, J. H., & Wang, J. P. (2014). Broad specific enzyme-linked immunosorbent assay for determination of residual phenothiazine drugs in swine tissues. *Analytical Biochemistry*, *454*(1), 7–13.

Geng, Z., Zhang, X., Fan, Z., Lv, X., Su, Y., & Chen, H. (2017). Recent progress in optical biosensors based on smartphone platforms. *Sensors*, *17*(11), 1–19.

Ghaleb, K., Afifi, M., & El-Gohary, M. (2013). Assessment of diagnostic techniques of urinary tuberculosis. *Mediterranean Journal of Hematology and Infectious Diseases*, *5*(1), 1–7.

Gkaravelaa, L., Papadimitriou-Olivgerisb, M., Fokaa,A., Kolonitsioua, F., Spiliopouloua, A., Charokoposc, N., Voulgaridisd, A., Tsiamitad, M., Marangosb, M., Evangelos, D., & Anastassioua, I. S. (2017). Combination of commercially available molecular assays and culture based methods in diagnosis of. *Brazilian Journal of Microbiology*, *48*(4), 785–790.

Glikmann, G., Mordhorst, C. H., & Koch, C. (1995). Monoclonal antibodies for the direct detection of influenza-A virus by ELISA in clinical specimens from patients with respiratory infections. *Clinical and Diagnostic Virology*, *3*(4), 361–369.

Gnoth, C., & Johnson, S. (2014, July). Strips of Hope: Accuracy of Home Pregnancy Tests and New Developments. *Geburtshilfe Und Frauenheilkunde*. Stuttgart · New York.

Govindarajan, K. K., & Chai, F. Y. (2011). BCG Adenitis—Need for Increased Awareness. *The Malaysian Journal of Medical Sciences*, *18*(2), 66–69.

Gradmann, C. (2006). Robert Koch and the white death : from tuberculosis to tuberculin. *Microbes and Infection*, *8*, 294–301.

Guerrero, S., Martínez-García, G., Serafín, V., & Agüí, L. P. Y.S. (2015). Electrochemical immunosensor for sensitive determination of the anorexigen peptide YY at grafted reduced graphene oxide electrode platforms S. *Analyst*, 1–18.

Gui, R., Huang, R., Nie, X.-M., Zhang, J.-H., Wen, X.-H., & Liu, J. (2014). Ultrasensitive detection based on gold nanoparticles for the platelet-associated tissue factor in patients with thrombotic diseases. *Analytical Methods*, *6*, 1–5.

Guilbault, G. G., Palleschi, G., & Lubrano, G. (1995). Non-invasive biosensors in clinical analysis. *Biosensors and Bioelectronics*, *10*(3–4), 379–392.

Güner, A., Çevik, E., & Mehmet, S. (2017). An electrochemical immunosensor for sensitive detection of Escherichia coli O157 : H7 by using chitosan , MWCNT , polypyrrole with gold nanoparticles hybrid sensing platform. *Food Chesmistry*, *229*, 358–365.

Guo, L., Jackman, J. A., Yang, H., Chen, P., Cho, N., & Kim, D. (2015).

Strategies for enhancing the sensitivity of plasmonic nanosensors. *Nano Today*, *10*(2), 213–239.

Gutierrez, M.C., Brisse, S., Brosch, R., Fabre, M., Omais, B., Marmiesse, M., Supply, P. & Vincent, V. (2005) Ancient origin and gene mosaicism of the progenitor of Mycobacterium tuberculosis. *PLos Pathog, 1(1)*, e5.

Guo, S., Xue, R., Li, Y., Wang, S. M., Ren, L., & Xu, J. J. (2012). The CFP10/ESAT6 complex of Mycobacterium tuberculosis may function as a regulator of macrophage cell death at different stages of tuberculosis infection. *Medical Hypotheses*, *78*(3), 389–392.

Haes, A. J., Chang, L., Klein, W. L., & Van Duyne, R. P. (2005). Detection of a Biomarker for Alzheimer’s Disease from Synthetic and Clinical Samples Using a Nanoscale Optical Biosensor. *Journal of the American Chemical Society*, *127*(7), 2264–2271.

Hans, R., & Marwaha, N. (2014). Nucleic acid testing-benefits and constraints. *Asian Journal of Transfusion Science*. India: Medknow Publications & Media Pvt Ltd.

Haque, F., Rahman, M. S., Ahmed, E., Bakshi, P. K., & Shaikh, A. A. (2013). A Cyclic Voltammetric Study of the Redox Reaction of Cu (II) in Presence of Ascorbic Acid in Different pH Media. *Dhaka. Univ. J. Sci.*, *61*(2), 161–166.

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

Hassani, A., Gauvreau, B., Fehri, M. F., Kabashin, A., & Skorobogatiy, M. (2008). Photonic Crystal Fiber and Waveguide-Based Surface Plasmon Resonance Sensors for Application in the Visible and Near-IR. *Electromagnetics*, *28*(3), 198–213.

Hemben, A., Ashley, J., & Tothill, I. E. (2017). Development of an Immunosensor for Pf HRP 2 as a biomarker for malaria detection. *Biosensors*, *7*(28), 1–14.

Hepple, P., Nguele, P., Greig, J., Bonnet, M., & Sizaire, V. (2010). Direct microscopy versus sputum cytology analysis and bleach sedimentation for diagnosis of tuberculosis: A prospective diagnostic study. *BMC Infectious Diseases*, *10*(276), 1–7.

Herrera, V., Perry, S., Parsonnet, J., & Banaei, N. (2011). Clinical application and limitations of interferon-γ release assays for the diagnosis of latent tuberculosis infection. *Clinical Infectious Diseases*, *52*(8), 1031–1037.

Hlywka, J. J., Hefle, S. L., & Taylor, S. L. (2000). A sandwich enzyme-linked immunosorbent assay for the detection of almonds in foods. *Journal of Food Protection*, *63*(2), 252–257.

Hnaiein, M., Hassen, W. M., Abdelghani, A., Fournier-Wirth, C., Coste, J., Bessueille, F., Jaffrezic-Renault, N. (2008). A conductometric immunosensor based on functionalized magnetite nanoparticles for *E. coli* detection. *Electrochemistry Communications*, *10*(8), 1152–1154.

Homola, J. (2008). Surface Plasmon Resonance Sensors for Detection of Chemical and Biological Species. *Chemical Reviews*, *108*(2), 462–493.

Hong, S. C., Chen, H., Lee, J., Park, H.-K., Kim, Y. S., Shin, H.-C., Lee, J. (2011a). Ultrasensitive immunosensing of tuberculosis CFP-10 based on SPR spectroscopy. *Sensors and Actuators B: Chemical*, *156*(1), 271–275.

Hong, S. C., Chen, H., Lee, J., Park, H. K., Kim, Y. S., Shin, H. C., Lee, J. (2011b). Ultrasensitive immunosensing of tuberculosis CFP-10 based on SPR spectroscopy. *Sensors and Actuators, B: Chemical*, *156*(1), 271–275.

Hong, S. C., Lee, J., Shin, H. C., Kim, C. M., Park, J. Y., Koh, K., Lee, J. (2011). Clinical immunosensing of tuberculosis CFP-10 in patient urine by surface plasmon resonance spectroscopy. *Sensors and Actuators, B: Chemical*, *160*(1), 1434–1438.

Hong, S., Choi, S., Do, H., & Hong, S. (2009). Biosensors and Bioelectronics Development of QCM biosensor to detect a marine derived pathogenic bacteria Edwardsiella tarda using a novel immobilisation method. *Biosensors and Bioelectronics*, *24*, 1635–1640.

Hsieh, S., Chang, C., Lu, C., Wei, C., Lin, C., Lai, H., & Lin, C. (2012). Rapid identification of Mycobacterium tuberculosis infection by a new array format-based surface plasmon resonance method. *Nanoscale Research Letters*, *7*(1), 180.

Huang, C.-J., Dostalek, J., Sessitsch, A., & Knoll, W. (2011). Long-Range Surface Plasmon-Enhanced Fluorescence Spectroscopy Biosensor for Ultrasensitive Detection of E. coli O157:H7. *Analytical Chemistry*, *83*(3), 674–677.

Huang, H., Li, J., Shi, S., Yan, Y., & Zhang, M. (2015). Detection of interferon-gamma for latent tuberculosis diagnosis using an immunosensor based on CdS quantum dots coupled to magnetic beads as labels. *International Journal of Electrochemical Science*, *10*, 2580–2593.

Hurt, L. (2003). *A founding father of biology* (Vol. 10).

Imperiale, B. R., Cataldi, Á. A., & Morcillo, N. S. (2017). In vitro anti-tuberculosis activity of azole drugs against Mycobacterium tuberculosis clinical isolates. *Revista Argentina de Microbiología*, *49*(4), 332–338.

Ivanov, M. R., Bednar, H. R. and Haes, A. J. (2009). Investigations of the mechanism of gold nanoparticle stability and surface functionalization in capillary electrophoresis. *ACS Nano*, 3 (2) 386-394.

Jana, N. R., Gearheart, L., and Murphy, C. J. (2001). Evidence for seedmediated nucletion in the chemical reduction of gold salts to gold nanoparticles. *Chem. Mater*, 13 (7) 2313-2322.

Jaramillo, M., Montagut, Y. J., Montoya, A., Robledo, J., Marin, P. A., Betancur, J. E., Torres, R. A. (2017). Design of a piezoelectric immunosensor for tuberculosis biomarker detection. *Pan American Health Care Exchanges (PAHCE)*, 1–7.

Jiang, X., Li, D., Xu, X., Ying, Y., Li, Y., Ye, Z., & Wang, J. (2008). Immunosensors for detection of pesticide residues. *Biosensors and Bioelectronics*, *23*(11), 1577–1587.

Jiang, Y., Liu, H., Wang, H., Dou, X., Zhao, X., Bai, Y.,Wan, K. (2013). Polymorphism of Antigen MPT64 in, *51*(5), 1558–1562.

Jnawali, H.N. & Ryoo, S. (2013). *Tuberculosis - Current Issues in Diagnosis and Management: First– and Second–Line Drugs and Drug Resistance*. (M. G. Mahboub, Bassam H., Vats, Ed.) (Chapter 10). Rijeka: InTech.

Jonge, M. I. De, Fretz, M. M., Romain, F., Bottai, D., Brodin, P., Honore, N., Brosch, R. (2007). ESAT-6 from Mycobacterium tuberculosis Dissociates from Its Putative Chaperone CFP-10 under Acidic Conditions and Exhibits Membrane-Lysing Activity □. *Journal of Bacteriology*, *189*(16), 6028–6034.

Justino, C. I. L., Duarte, A. C., & Rocha-Santos, T. A. P. (2016). Immunosensors in Clinical Laboratory Diagnostics. *Advances in Clinical Chemistry*, *73*, 65–108.

Kashyap, R. S., Rajan, A. N., Ramteke, S. S., Agrawal, V. S., Kelkar, S. S., Purohit, H. J., Daginawala, H. F. (2007). Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study. *BMC Infectious Diseases*, *7*(74), 1–6.

Kaushik, A., Yndart, A., Kumar, S., Jayant, R. D., Vashist, A., Brown, A. N., Nair, M. (2018). A sensitive electrochemical immunosensor for label-free detection of Zika-virus protein. *Nature*, *8*(9700), 3–7.

Kendall, E. A. (2018, March 28). Tuberculosis in children: under-counted and under-treated.

Khurshid, S., Afzal, M., Khalid, R., & Akhtar, M. W. (2017). Potential of multi-component antigens for tuberculosis diagnosis. *Biologicals*, *48*, 109–113.

Kidenya, B. R., Mshana, S. E., Gerwing-adima, L., Kidola, J., & Kasang, C. (2017). International Journal of Infectious Diseases Drug adherence and ef fi cacy of smear microscopy in the diagnosis of pulmonary tuberculosis after 2 months of medication in. *International Journal of Infectious Diseases*, *63*, 43–47.

Kim, J., Hong, S. C., Hong, J. C., Chang, C. L., Park, T. J., Kim, H.-J., & Lee, J. (2015a). Clinical immunosensing of tuberculosis CFP-10 antigen in urine using interferometric optical fiber array. *Sensors and Actuators B: Chemical*, *216*, 184–191.

Kim, J., Hong, S. C., Hong, J. C., Chang, C. L., Park, T. J., Kim, H. J., & Lee, J. (2015b). Clinical immunosensing of tuberculosis CFP-10 antigen in urine using interferometric optical fiber array. *Sensors and Actuators, B: Chemical*, *216*, 184–191.

Kim, J., Lee, J., Lee, K. I., Park, T. J., & Kim, H. J. (2013). *Rapid monitoring of CFP-10 during culture of Mycobacterium tuberculosis by using a magnetophoretic immunoassay*. *Sensors and Actuators B-Chemical* (Vol. 177).

Kirkman, H. N., & Gaetani, G. F. (1984). Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. *Proceedings of the National Academy of Sciences of the United States of America*, *81*(July), 4343–4347.

Kobayashi, M., Ray, S. M., Hanfelt, J., & Wang, Y. F. (2014). Diagnosis of tuberculosis by using a nucleic acid amplification test in an urban population with high HIV prevalence in the United States. *PLoS ONE*, *9*(10), 1–7.

Koestel, C., Simonin, C., Belcher, S., & Rösti, J. (2016). Implementation of an Enzyme Linked Immunosorbent Assay for the Quantification of Allergenic Egg Residues in Red Wines Using Commercially Available Antibodies. *Journal of Food Science*, *81*(8), T2099–T2106.

Korimbocus, J., Dehay, N., Tordo, N., & Morgeaux, S. (2016). Development and validation of a quantitative competitive ELISA for potency testing of equine anti rabies sera with other potential use. *Vaccine*, *34*, 3310–3316.

Kosaka, P. M., Pini, V., Ruz, J. J., da Silva, R. A., González, M. U., Ramos, D., Tamayo, J. (2014). Detection of cancer biomarkers in serum using a hybrid mechanical and optoplasmonic nanosensor. *Nature Nanotechnology*, *9*, 1047.

Kozma, P., Kehl, F., Ehrentreich-Förster, E., Stamm, C., & Bier, F. F. (2014). Integrated planar optical waveguide interferometer biosensors: A comparative review. *Biosensors and Bioelectronics*, *58*, 287–307.

Krajewska, A., Radecki, J., & Radecka, H. (2008). A voltammetric biosensor based on glassy carbon electrodes modified with single-walled carbon nanotubes/hemoglobin for detection of acrylamide in water extracts from potato crisps. *Sensors*, *8*(9), 5832–5844.

Kruczak, K., Mastalerz, L., & Krzysztof, S. (2016). Interferon-gamma release assays and tuberculin skin testing for diagnosing latent Mycobacterium tuberculosis infection in at-risk groups in Poland. *International Journal of Mycobacteriology*, *5*, 27–33.

Kruss, S., Hilmer, A. J., Zhang, J., Reuel, N. F., Mu, B., & Strano, M. S. (2013). Carbon nanotubes as optical biomedical sensors. *Advanced Drug Delivery*

Reviews, *65*(15), 1933–1950.

Kuno, G., Gomez, I., & Gubler, D. J. (1991). An ELISA procedure for the diagnosis of dengue infections. *Journal of Virological Methods*, *33*(1–2), 101–113.

Lakshmipriya, T., Gopinath, S. C. B., & Tang, T.-H. (2016). Biotin-Streptavidin Competition Mediates Sensitive Detection of Biomolecules in Enzyme Linked Immunosorbent Assay. *Plos One*, *11*(3), 1–14.

Lan, W., Chen, G., Cui, F., Tan, F., Liu, R., & Yushupujiang, M. (2012). Development of a novel optical biosensor for detection of organophoshorus pesticides based on methyl parathion hydrolase immobilized by metal-chelate affinity. *Sensors*, *12*(7), 8477–8490.

Lan, Y., Wang, S., Yin, Y., Hoffmann, W. C., & Zheng, X. (2008). Using a Surface Plasmon Resonance Biosensor for Rapid Detection of Salmonella Typhimurium in Chicken Carcass. *Journal of Bionic Engineering*, *5*(3), 239–246.

Laraque, F., Griggs, A., Slopen, M., & Munsiff, S. S. (2009). Performance of Nucleic Acid Amplification Tests for Diagnosis of Tuberculosis in a Large Urban Setting. *Clinical Infectious Diseases*, *49*(1), 46–54.

Lawn, S. D., & Nicol, M. P. (2011). Xpert ® MTB / RIF assay : development , evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol.*, *6*(9), 1067–1082.

Lechuga, L. (2005). Optical biosensors. *Comprehensive Analytical Chemistry*, *44*, 209–250.

Lei, K. F., & Leung, P. H. M. (2012). Microelectrode array biosensor for the detection of Legionella pneumophila. *Microelectronic Engineering*, *91*, 174–177.

Leirs, K., Tewari Kumar, P., Decrop, D., Pérez-Ruiz, E., Leblebici, P., Van Kelst, B., Spasic, D. (2016). Bioassay Development for Ultrasensitive Detection of Influenza A Nucleoprotein Using Digital ELISA. *Analytical Chemistry*, *88*(17), 8450–8458.

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*, *2*, 2–10.

Li, M., Li, Y., Li, D., & Long, Y. (2012). Analytica Chimica Acta Recent developments and applications of screen-printed electrodes in environmental assays — A review. *Analytica Chimica Acta*, *734*, 31–44.

Li, N., Huang, X., Sun, D., Yu, W., Tan, W., Luo, Z., & Chen, Z. (2018). Dual-aptamer-based voltammetric biosensor for the Mycobacterium tuberculosis antigen MPT64 by using a gold electrode modified with a peroxidase loaded composite consisting of gold nanoparticles and a Zr (IV)/ terephthalate metal-organic framework. *Microchimica Acta*, *2*, 1–7.

Liang, J., Yao, C., Li, X., Wu, Z., Huang, C., Fu, Q., Tang, Y. (2015). Silver nanoprism etching-based plasmonic ELISA for the high sensitive detection of prostate-specific antigen. *Biosensors and Bioelectronics*, *69*, 128–134.

Liddell, E. (2013). Chapter 3.1 - Antibodies. In D. Wild (Ed.), *The Immunoassay Handbook (Fourth Edition)* (Fourth Edi, pp. 245–265). Oxford: Elsevier.

Lin, A. V. (2015). Direct ELISA. In R. Hnasko (Ed.), *ELISA: Methods and Protocols* (pp. 61–67). New York, NY: Springer New York.

Liu, C., Dong, J., & Waterhouse, G. I. N. (2017). Electrochemical immunosensor with nanocellulose-Au composite assisted multiple signal amplification for detection of avian leukosis virus subgroup J. *Biosensors and Bioelectronic*, 1–25.

Liu, C., Zhao, Z., Fan, J., Lyon, C. J., Wu, H.-J., Nedelkov, D., Hu, Y. (2017). Quantification of circulating *Mycobacterium tuberculosis* antigen peptides allows rapid diagnosis of active disease and treatment monitoring. *Proceedings of the National Academy of Sciences*, *114*(15), 3969–3974.

Liu, J., Zanardi, S., Powers, S., & Suman, M. (2012). Development and practical application in the cereal food industry of a rapid and quantitative lateral flow immunoassay for deoxynivalenol. *Food Control*, *26*(1), 88–91.

Liu, L. B., & Saltman, M. A. (1996). Immunosensor Technology. *Laboratory Medicine*, *27*(2), 109–115.

Liu, L., Zhao, Z., Cai, M., Jiang, X., Kang, Y., Dai, Q., Xie, G. (2016). Electrochemical Determination of 16s Ribosomal RNA of Mycobacterium Tuberculosis Using Magnetite on Silica with DNA-Functionalized Gold Nanoparticles AU - Sheng, Shangchun. *Analytical Letters*, *49*(9), 1379–1387.

Liu, X., Hu, Y., Zheng, S., Liu, Y., He, Z., & Luo, F. (2016). Sensors and Actuators B : Chemical Surface plasmon resonance immunosensor for fast , highly sensitive , and in situ detection of the magnetic nanoparticles-enriched Salmonella enteritidis. *Sensors & Actuators: B. Chemical*, *230*, 191–198.

Liu, X., & Jiang, H. (2017). Construction and potential applications of biosensors for proteins in clinical laboratory diagnosis. *Sensors*, *17*(12), 1–23.

Liu, Y., Tuleouva, N., Ramanculov, E., & Revzin, A. (2010). Aptamer-Based Electrochemical Biosensor for Interferon Gamma Detection. *Analytical Chemistry*, *82*(19), 8131–8136.

Long, F., He, M., Shi, H. C., & Zhu, A. N. (2008). Development of evanescent wave all-fiber immunosensor for environmental water analysis. *Biosensors and Bioelectronics*, *23*(7), 952–958.

Lu, C.-Y., Chang, L.-Y., Chen, P.-J., Xia, N.-S., Shao, P.-L., & Huang, L.-M. (2012). A highly specific ELISA for diagnosis of 2009 influenza A (H1N1) virus infections. *Journal of the Formosan Medical Association*, *111*(12), 693–697.

Lu, H. T. (2013). Synthesis and Characterization of Amino Functionalized. *Colloid Journal*, *75*(3), 311–312.

Luca, S., & Mihaescu, T. (2013). History of BCG Vaccine. *Mædica*, *8*(1), 53–58. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3749764/

de Oliveira, L. F., Picco, S. A., Larissa B. C., Kaliandra A. G., d João H. Z., dos Santos, J. K., and Mateus B. C. L. (2017). Tailored Silica Nanoparticles Surface to Increase Drug Load and Enhance Bactericidal Response. *Journal Braz. Chem. Soc.*, *28*(9), 1715–1724.

Luppa, P. B., Sokoll, L. J., & Chan, D. W. (2001a). Immunosensors—principles and applications to clinical chemistry. *Clinica Chimica Acta*, *314*(1–2), 1–26.

Luppa, P. B., Sokoll, L. J., & Chan, D. W. (2001b). Immunosensors—principles and applications to clinical chemistry. *Clinica Chimica Acta*, *314*(1–2), 1–26.

Mahalingam, V., Onclin, S., Ravoo, B. J., Huskens, J., & Reinhoudt, D. N. (2004). Directed Self-Assembly of Functionalized Silica Nanoparticles on Molecular Printboards through Multivalent Supramolecular Interactions, (27), 11756–11762.

Malhotra, B. D., & Chaubey, A. (2003). Biosensors for clinical diagnostics industry. *Sensors and Actuators B: Chemical*, *91*(1–3), 117–127.

Malinski, T., Kubaszewski, E., & Kiechle, F. (1996). *Electrochemical and Spectroscopic Methods of Nitric Oxide Detection*. *Nitric Oxide Synthase: Characterization and Functional Analysis* (Vol. 31). Elsevier Masson SAS.

Marais, B. J., Graham, S. M., Cotton, M. F., & Beyers, N. (2007). Diagnostic and management challenges for childhood tuberculosis in the era of HIV. *The Journal of Infectious Diseases*, *196*, S76-85.

Martins, T. D., Ribeiro, A. C. C., Camargo, H. S. de, Filho, P. A. da C., Cavalcante, H. P. M., & Dias, D. L. (2013). New Insights on Optical Biosensors: Techniques, Construction and Application. In T. Rinken (Ed.), *State of the Art in Biosensors - General Aspects*. InTech.

Martynov, A.V., Bomko, T.V.N. T., & Lisnyak, Y.V., Romanova, E.A., Kabluchko, T.V., Sidorenko, T.A., Igumnova, N.I., Pogorelaya, M .S., Shcherbak, E.M., Yukhimenko, V.I., Farber, B.S. F. S. (2016). Tuberculosis As an Infectious Pathology of Immune System. *Annals of Mechnikov Institute*, 8–14.

McNerney, R., Maeurer, M., Abubakar, I., Marais, B., McHugh, T. D., Ford, N., Zumla, A. (2012). Tuberculosis diagnostics and biomarkers: Needs, challenges, recent advances, and opportunities. *Journal of Infectious Diseases*, *205*(2), 1–12.

Mehrotra, P. (2016). Biosensors and their applications - A review. *Journal of Oral Biology and Craniofacial Research*, *6*(2), 153–159.

Mello, L. D., & Kubota, L. T. (2002). Review of the use of biosensors as analytical tools in the food and drink industries. *Food Chemistry*, *77*(2), 237–256.

Mirhosseini, S. A., Ali, A., Fooladi, I., Amani, J., & Sedighian, H. (2017). Production of recombinant flagellin to develop ELISA-based detection of Salmonella Enteritidis. *Brazilian Journal of Microbiology*, *48*(4), 774–781.

Mistry, K. K., Layek, K., Chell, T. N., Chaudhuri, C. R., & Saha, H. (2016). Design and development of an amperometric immunosensor based on screen-printed electrodes. *Anal. Methods*, *8*(15), 3096–3101.

Mohamad, F. S., Mat Zaid, M. H., Abdullah, J., Zawawi, R. M., Lim, H. N., Sulaiman, Y., & Abdul Rahman, N. (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*(12).

Mohammed, M.-I., & Desmulliez, M. P. Y. (2011). Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review. *Lab Chip*, *11*(4), 569–595.

Moina C. and Ybarra G. (2012). Fundamentals and Applications of Immunosensors, Advances in Immunoassay Technology.

Montoya, A., March, C., Montagut, Y. J., Moreno, M. J., Manclus, J. J., Arnau, A., Torres, P. A. M. and R. A. (2017). A High Fundamental Frequency (HFF)-based QCM Immunosensor for Tuberculosis Detection. *Current Topics in Medicinal Chemistry*.

Mouli, C., Tiwari, I., Nand, V., Sood, K. N., Sumana, G., & Dhar, B. (2017). Sensors and Actuators B : Chemical Highly sensitive electrochemical immunosensor based on graphene-wrapped copper oxide-cysteine hierarchical structure for detection of pathogenic bacteria. *Sensors & Actuators: B. Chemical*, *238*, 1060–1069.

Muhamuda, K., Madhusudana, S. N., & Ravi, V. (2007). Development and evaluation of a competitive ELISA for estimation of rabies neutralizing antibodies after post-exposure rabies vaccination in humans. *International Journal of Infectious Diseases*, *11*(5), 441–445.

Mukhopadhyay, S., & Balaji, K. N. (2011). The PE and PPE proteins of Mycobacterium tuberculosis. *Tuberculosis*, *91*(5), 441–447.

Mukundan, H., Kumar, S., Price, D. N., Ray, S. M., Lee, Y.-J., Min, S., Swanson, B. I. (2012). Rapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor. *Tuberculosis*, *92*(5), 407–416.

Nagel, T., Ehrentreich-Förster, E., Singh, M., Schmitt, K., Brandenburg, A., Berka, A., & Bier, F. F. (2008). Direct detection of tuberculosis infection in blood serum using three optical label-free approaches. *Sensors and Actuators B: Chemical*, *129*(2), 934–940.

Nagy, Z. A. (2014). Chapter 4 - Monoclonal Antibodies: The Final Proof for Clonal Selection. In Z. A. Nagy (Ed.), *A History of Modern Immunology* (pp. 33–40). Amsterdam: Academic Press.

Narayan, R., Raja, S., Kumar, S., Sambasivam, M., Jagadeesan, R., Arunagiri, K., Palani, G. (2016, July). A novel indirect ELISA for diagnosis of dengue fever. *The Indian Journal of Medical Research*. India.

Nehl, C. L., & Hafner, J. H. (2008). Shape-dependent plasmon resonances of gold nanoparticles. *Journal of Materials Chemistry*, *18*(21), 2415–2419.

Ng, B. (2014, January 15). TB kills more annually than dengue. *Malay Mail Online*, pp. 1–2. Retrieved from http://www.themalaymailonline.com/malaysia/article/tb-kills-more-annually-than-dengue-says-ministry#Zs8LAFQ417FbzQAf.97

Nie, X.-M., Huang, R., Dong, C.-X., Tang, L.-J., Gui, R., & Jiang, J.-H. (2014). Plasmonic ELISA for the ultrasensitive detection of Treponema pallidum. *Biosensors & Bioelectronics*, *58*, 314–319.

Nieto, D., Martínez-guinó, L., Jiménez-melsió, A., Segalés, J., & Kekarainen, T. (2015). Development of an indirect ELISA assay for the detection of IgG antibodies against the ORF1 of Torque teno sus viruses 1 and 2 in conventional pigs. *Veterinary Microbiology*, *180*(1–2), 22–27.

Nordlee, J. A., Hefle, S. L., & Taylor, S. L. (1996). A sandwich enzyme-linked immunosorbent assay (ELISA) for the quantitation of peanut in foods. *Book of Abstracts: 1996 IFT Annual Meeting*, *57*(5), 28.

Nouri, A., Ahari, H., & Shahbazzadeh, D. (2017). Designing a direct ELISA kit for the detection of Staphylococcus aureus enterotoxin A in raw milk samples. *International Journal of Biological Macromolecules*, 1–6.

Okafor, C., Grooms, D., Alocilja, E., & Bolin, S. (2008). Fabrication of a Novel Conductometric Biosensor for Detecting Mycobacterium avium subsp. paratuberculosis Antibodies. *Sensors*, *8*(9), 6015–6025.

Pai, M., Denkinger, C. M., Kik, S. V., Rangaka, M. X., Zwerling, A., Oxlade, O., Banaei, N. (2014). Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clinical Microbiology Reviews*, *27*(1), 3–20.

Pai, M., Kalantri, S., & Dheda, K. (2006). New tools and emerging technologies for the diagnosis of tuberculosis: Part I. *Expert Review of Molecular Diagnostics*, *6*(3), 413–422.

Pal, S., Dauner, A. L., Mitra, I., Forshey, B. M., Garcia, P., Morrison, A. C., Wu, S.-J. L. (2014). Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. *PLoS ONE*, *9*(11), e113411.

Pang, Y., Zhao, A., Cohen, C., Kang, W., Lu, J., Wang, G., Zheng, S. (2016). Current status of new tuberculosis vaccine in children. *Human Vaccines & Immunotherapeutics*, *12*(4), 960–970.

Pariwono, A. M., Lo, T., Lim, C. S., Wang, S. X., & Chan, Y. W. (2007). Rapid Tuberculosis Detection Technique for On-site Patient Screening. *Journal of Biomedical & Pharmaceutical Engineering*, *1*, 27–33.

Parsons, L. M., Gutierrez, C., Lee, E., Paramasivan, C. N., Abimiku, A., Spector, S., Nkengasong, J. (2011). Laboratory Diagnosis of Tuberculosis in Resource-Poor Countries : Challenges and Opportunities. *Clinical Microbiology Reviews*, *24*(2), 314–350.

Pasternack, R. M., Amy, S. R., & Chabal, Y. J. (2008). Attachment of 3- (Aminopropyl) triethoxysilane on Silicon Oxide Surfaces : Dependence on Solution Temperature. *Langmuir*, *24*(9), 12963–12971.

Pedersen, M. H., Holzhauser, T., Bisson, C., Conti, A., Jensen, L. B., Skov, P. S., Poulsen, L. K. (2008). Soybean allergen detection methods--a comparison study. *Molecular Nutrition & Food Research*, *52*(12), 1486–1496.

Peik-see, T., Pandikumar, A., Nay-ming, H., Hong-ngee, L., & Sulaiman, Y. (2014). Simultaneous Electrochemical Detection of Dopamine and Ascorbic Acid Using an Iron Oxide/Reduced Graphene Oxide Modified Glassy Carbon Electrode, 15227–15243.

Perepelytsina, O., Yakymchuk, O., V Sydorenko, M., N Bakalinska, O., Bloisi, F., & Vicari, L. (2016). Functionalization of Carbon Nanomaterial Surface by Doxorubicin and Antibodies to Tumor Markers. *Nanoscale Research Letters*, *11*(314), 1–13.

Petryayeva, E., & Krull, U. J. (2011). Localized surface plasmon resonance: Nanostructures, bioassays and biosensing—A review. *Analytica Chimica Acta*, *706*(1), 8–24.

Piliarik, M., Vaisocherová, H., & Homola, J. (2005). A new surface plasmon resonance sensor for high-throughput screening applications. *Biosensors and Bioelectronics*, *20*(10), 2104–2110.

Pitre, K., & Tiwari, S. (2011). Microfaradaic Electrochemical Biosensors for the Study of Anticancer Action of DNA Intercalating Drug: Epirubicin. In P. A. Serra (Ed.), *Biosensors for Health, Environment and Biosecurity*. Rijeka: InTech.

Podrouzek, P., Krabec, Z., Mancal, P., & Presl, J. (1988). The development and evaluation of Sevatest ELISA hCG Micro I. kit as a test for pregnancy. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, *32*(4), 467–476.

Pohanka, M., & Republic, C. (2008). Electrochemical biosensors – principles and applications. *Journal of Applied Biomedicine*, *6*(2), 57–64.

Polynkin, P., Polynkin, A., Peyghambarian, N., & Mansuripur, M. (2005). Evanescent field-based optical fiber sensing device for measuring the refractive index of liquids in microfluidic channels. *Opt. Lett.*, *30*(11), 1273–1275.

Prabha, P., & Kocher, D. K. (2013). Dot Elisa : Immunological Technique for Pesticide Residue Analysis Immunogen / Hapten Preparation. *Research Journal of Recent Sciences*, *2*, 5–11.

Prakash, K., Prajapati, S., Ahmad, A., Jain, S. K., & Bhakuni, V. (2002). Unique oligomeric intermediates of bovine liver catalase. *Protein Science : A Publication of the Protein Society*, *11*(1), 46–57.

Prakash, P. A., Yogeswaran, U., & Chen, S.-M. (2009). A Review on Direct Electrochemistry of Catalase for Electrochemical Sensors. *Sensors*.

Proll, G., Markovic, G., Steinle, L., & Gauglitz, G. (2009). Reflectometric Interference Spectroscopy. In A. Rasooly & K. E. Herold (Eds.), *Biosensors and Biodetection* (pp. 167–178). Totowa, NJ: Humana Press.

Qie, Z., Ning, B., Liu, M., Bai, J., Peng, Y., Song, N., Gao, Z. (2013). Fast detection of atrazine in corn using thermometric biosensors. *Analyst*, *138*(17), 5151–5156.

Quenard, F., Edouard, P., Drancourt, M., & Brouqui, P. (2017). International Journal of Antimicrobial Agents Role of second-line injectable antituberculosis drugs in the treatment of MDR / XDR tuberculosis. *International Journal of Antimicrobial Agents*, *50*(2), 252–254.

Radi, A.-E., Muñoz-Berbel, X., Lates, V., & Marty, J.-L. (2009). Label-free impedimetric immunosensor for sensitive detection of ochratoxin A. *Biosensors and Bioelectronics*, *24*(7), 1888–1892.

Radi, A., & Elmogy, T. (2005). Differential pulse voltammetric determination of carvedilol in tablets dosage form using glassy carbon electrode. *IL FARMACO*, *60*, 43–46.

Rahman, S. A., Ariffin, N., Yusof, N. A., & Abdullah, J. (2017). Thiolate-Capped CdSe/ZnS Core-Shell Quantum Dots for the Sensitive Detection of Glucose. *Sensors*, *17*(1537), 1–12.

Rajender, T. (2010). Strong Growth Predicted for Biosensors Market. Retrieved March 28, 2018, from https://www.sensorsmag.com/components/strong-growth-predicted-for-biosensors-market

Ramaraj, T., Angel, T., Dratz, E. A., Jesaitis, A. J., & Mumey, B. (2012). Antigen-antibody interface properties: Composition, residue interactions, and features of 53 non-redundant structures. *Biochimica et Biophysica Acta - Proteins and Proteomics*, *1824*(3), 520–532.

Ramírez, N. B., Salgado, A. M., & Valdman, B. (2009). The evolution and developments of immunosensors for health and environmental monitoring: Problems and perspectives. *Brazilian Journal of Chemical Engineering*, *26*(2), 227–249.

Torati, R. S., Venu, R., Yoon, S., & Kim, C. (2015, December 1). Electrochemical Biosensor for Mycobacterium Tuberculosis DNA Detection Based on Gold Nanotubes Array Electrode Platform. *Biosensors and Bioelectronics*.

Rao, P. V. R., Murthy, M. K., Basirudeen, S., Sharma, P., Swaminathan, S., & Raja, A. (2009). Improved diagnosis of tuberculosis in HIV-positive patients using RD1-encoded antigen CFP-10. *International Journal of Infectious Diseases*, *13*(5), 613–622.

Rashtbari, S., Dehghan, G., Yekta, R., & Jouyban, A. (2017). Investigation of the binding mechanism and inhibition of bovine liver catalase by quercetin: Multi-spectroscopic and computational study. *BioImpacts : BI*, *7*(3), 147–153.

Rastogi, N, Legrand, E., & Sola, C. (2001). The mycobacteria : an introduction to nomenclature and pathogenesis . *Rev. Sci. Tech. Off. Int. Epiz*, *20*(1), 20–54.

Ravidra, N. M., Prodan, C., Fnu, S., Padronl, I., & Sikha, S. K. (2007). Advances in the manufacturing types, and applications of biosensors. *Jom*, *59*(12), 37–43.

Reddy, J. R., Kwang, J., Lechtenberg, K. F., Khan, N.C. & Reddy, B. P. M. M. C. (2002). An immunochromatographic serological assay for the diagnosis of. *Comparative Immunology, Microbiology and Infectious Diseases*, *25*(1), 21–27.

Renshaw, P. S., Lightbody, K. L., Veverka, V., Muskett, F. W., Kelly, G., Frenkiel, T. A., Carr, M. D. (2005). Structure and function of the complex formed by the tuberculosis virulence factors CFP-10 and. *The EMBO Journal*, *24*(14), 2491–2498.

Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., Serra, P. A. (2016). Enzyme biosensors for biomedical applications: Strategies for

Rodrigo, G., Gruvegård, M., & Van Alstine, J. M. (2015). Antibody Fragments and Their Purification by Protein L Affinity Chromatography. *Antibodies*, *4*(3), 259–277.

Rodriguez-Mozaz, S., Lopez de Alda, M. J., & Barceló, D. (2007). Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography–mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. *Journal of Chromatography*

A, *1152*(1–2), 97–115. h

Rountree, K. J., Mccarthy, B. D., Rountree, E. S., Eisenhart, T. T., & Dempsey, J. L. (2017). A Practical Beginner ' s Guide to Cyclic Voltammetry. *Chemical Education*, *95*, 197–206.

Sabeta, C., & Ngoepe, E. (2015). Chapter Seven - Preparation of Fluorescent Antibody Conjugate in Goats. In C. Rupprecht & T. Nagarajan (Eds.), *Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention, Volume 2* (pp. 69–81). Academic Press.

Saengdee, P., Chaisriratanakul, W., Bunjongpru, W., Sripumkhai, W., Srisuwan, A., Hruanun, C., Promptmas, C. (2016). A silicon nitride ISFET based immunosensor for Ag85B detection of tuberculosis. *Analyst*, *141*(20), 5767–5775.

Sala, G., Cordioli, P., Moreno-Martin, A., Tollis, M., Brocchi, E., Piccirillo, A., & Lavazza, A. (2003). ELISA test for the detection of influenza H7 antibodies in avian sera. *Avian Diseases*, *47*(3 Suppl), 1057–1059.

Samarajeewa, U., Wei, C. I., Huang, T. S., & Marshall, M. R. (1991). Application of immunoassay in the food industry. *Critical Reviews in Food Science and Nutrition*, *29*(6), 403–434.

Sarkar, S., Tang, X. L., Das, D., Spencer, J. S., Lowary, T. L., & Suresh, M. R. (2012). A bispecific antibody based assay shows potential for detecting tuberculosis in resource constrained laboratory settings. *PLoS ONE*, *7*(2), 1–10.

Satija, J., Punjabi, N., Mishra, D., & Mukherji, S. (2016). Plasmonic-ELISA: expanding horizons. *RSC Adv.*, *6*(88), 85440–85456.

Scarpellini, P., Tasca, S., Galli, L., Beretta, A., Lazzarin, A., & Fortis, C. (2004). Selected pool of peptides from ESAT-6 and CFP-10 proteins for detection of Mycobacterium tuberculosis infection. *Journal of Clinical Microbiology*, *42*(8), 3469–3474.

Scharf, A., Kasel, U., Wichmann, G., & Besler, M. (2013). Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.). *Journal of Agricultural and Food Chemistry*, *61*(43), 10261–10272.

Schmitt, K., Oehse, K., Sulz, G., & Hoffmann, C. (2008). Evanescent field Sensors Based on Tantalum Pentoxide Waveguides – A Review. *Sensors (Basel, Switzerland)*, *8*(2), 711–738. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3927514/

Scholz, F. (2015). Voltammetric techniques of analysis : the essentials. *ChemTexts*, *1*(17), 1–24.

Schroeder, H. W., & Cavacini, L. (2010). Structure and function of immunoglobulins. *American Academy of Allergy, Asthma & Immunology*, *125*(32), s41–s52.

Schultz, J. S. (1987). Sensitivity and Dynamics of Bioreceptor-based Biosensors. *Annals of the New York Academy of Sciences*, *506*(1), 406–414.

Sengel, Y. T., Guler, E., Gumus, Z. P., Aldemir, E., Coskunol, H., Akbulut, H., Yagci, Y. (2017). An immunoelectrochemical platform for the biosensing of ‘Cocaine use.’ *Sensors and Actuators B: Chemical*, *246*, 310–318.

Sharma, D. C. (2017). New plan to end tuberculosis in south and southeast Asia. *The Lancet*, *389*(10075), 1183.

Shavanova, K., Bakakina, Y., Burkova, I., Shtepliuk, I., Viter, R., Ubelis, A., Khranovskyy, V. (2016). Application of 2D non-graphene materials and 2D oxide nanostructures for biosensing technology. *Sensors*, *16*(2), 1–23.

Shehzada, A., Rehmana, G., Ul-Islamb, M., & Khattakb, W.A. (2015). Review article Challenges in the development of drugs for the treatment of. *Brazilian Journal of Infectious Diseases*, *17*(1), 74–81.

Shi, J., Chan, C., Pang, Y., Ye, W., Tian, F., Lyu, J., Yang, M. (2015). A fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots (GQDs) and gold nanoparticles (AuNPs) for the detection of mecA gene sequence of Staphylococcus aureus. *Biosensors and Bioelectronics*, *67*, 595–600.

Shin, Y.-B., Jo, N., & joong Lee, K. (2015). Ultra-sensitive detection of biomarker using localized surface plasmon resonance (LSPR) enhanced by ELISA. *Progress in Biomedical Optics and Imaging - Proceedings of SPIE*, *9537*, 1–9.

Singh, B. K., Sharma, S. K., Sharma, R., Sreenivas, V., Myneedu, V. P., Kohli, M., Sarin, S. (2017). Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. *PLoS ONE*, *12*(8), 1–9.

Singh, K. K., Dong, Y., Hinds, L., Keen, M. A., Belisle, J. T., Zolla-Pazner, S., Laal, S. (2003). Combined use of serum and urinary antibody for diagnosis of tuberculosis. *The Journal of Infectious Diseases*, *188*(3), 371–377.

Singh, R., Mukherjee, M. Das, Sumana, G., Gupta, R. K., Sood, S., & Malhotra, B. D. (2014). Biosensors for pathogen detection: A smart approach towards clinical diagnosis. *Sensors and Actuators B: Chemical*, *197*, 385–404.

Smitha, S. L., Nissamudeen, K. M., Philip, D., & Gopchandran, K. G. (2008). Studies on surface plasmon resonance and photoluminescence of silver nanoparticles. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *71*(1), 186–190.

Soo, P. C., Horng, Y. T., Chen, A. T., Yang, S. C., Chang, K. C., Lee, J. J., & Peng, W. P. (2015). Validation of nanodiamond-extracted CFP-10 antigen as a biomarker in clinical isolates of Mycobacterium tuberculosis complex in broth culture media. *Tuberculosis*, *95*(5), 620–624.

Spasevska, I., Duong, M. N., Klein, C., & Dumontet, C. (2015). Advances in Bispecific Antibodies Engineering: Novel Concepts for Immunotherapies. *Journal of Blood Disorders & Transfusion*, *6*(1), 1–8.

Sreenivasa, K., El-hami, K., Kodaki, T., & Matsushige, K. (2005). A novel method for synthesis of silica nanoparticles, *289*, 125–131.

Srivastava, S. K. (2014). *Biosensor Based Detection of Tuberculosis Biomarkers*. Wageningen University.

Srivastava, S. K., Rijn, C. J. M. Van, & Jongsma, M. A. (2016). RSC Advances. *RSC Advances*, *6*(February), 17759–17771.

Srivastava, S. K., van Rijn, C. J. M., & Jongsma, M. A. (2016). Biosensor-based detection of tuberculosis. *RSC Adv.*, *6*(22), 17759–17771.

Steingart, K. R., Ng, V., Henry, M., Hopewell, P. C., Ramsay, A., Cunningham, J., Perkins, M. D. (2006). Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis : a systematic review. *Lancet Infect Dis*, *6*, 664–674.

Steve M. Blevins, M. S. B. (2010). Robert Koch and the ‘ golden age ’ of bacteriology. *Interntional Journal of Infectious Disease*, *14*, 744–751.

Stills, H. F. (2012). Chapter 11 - Polyclonal Antibody Production. In M. A. Suckow, K. A. Stevens, & R. P. Wilson (Eds.), *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* (pp. 259–274). Boston: Academic Press.

Strianese, M., Staiano, M., Ruggiero, G., Labella, T., Pellecchia, C., & D’Auria, S. (2012). Fluorescence-based biosensors. *Methods in Molecular Biology*, *875*, 193–216.

Su, X., & Li, Y. (2004). A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of Escherichia coli O157 : H7, *19*, 563–574.

Suraiya, S., Musa, M., Suppian, R. J. A. H. (2012). Asian Pacific Journal of Tropical Disease. *Asian Pacific Journal of Tropical Disease*, *2*(1), s312–s315.

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. *Biosens Bioelectron*, *1*(123), 141-151.

Tonjum, T., Klintz, L., Bergan, T., Baann, J., Furuberg, G., Cristea, M., Hoffner,

S. (1996). Direct detection of Mycobacterium tuberculosis in respiratory samples from patients in Scandinavia by polymerase chain reaction. *Clinical Microbiology and Infection*, *2*(2), 127–131.

Tama, L., Hansted, E., Vitkauskien, A., Miliauskas, S., Naud, A., & Brigita, Š. (2017). ScienceDirect Use of interferon-gamma release assay and tuberculin skin test in diagnosing tuberculosis in Lithuanian adults : A comparative analysis. *Medicina*, *53*, 159–165.

Tammam, S. N., Khalil, M. A. F., Abdul Gawad, E., Althani, A., Zaghloul, H., & Azzazy, H. M. E. (2017). Chitosan gold nanoparticles for detection of amplified nucleic acids isolated from sputum. *Carbohydrate Polymers*, *164*, 57–63.

Teles, F. S. R. R. (2011). Biosensors and rapid diagnostic tests on the frontier between analytical and clinical chemistry for biomolecular diagnosis of dengue disease: A review. *Analytica Chimica Acta*, *687*(1), 28–42.

Teo, J., Jureen, R., Chiang, D., Chan, D., & Lin, R. (2011). Comparison of two nucleic acid amplification assays, the Xpert MTB/RIF assay and the amplified mycobacterium tuberculosis direct assay, for detection of Mycobacterium tuberculosis in respiratory and nonrespiratory specimens. *Journal of Clinical Microbiology*, *49*(10), 3659–3662.

Thacker, J. D., Casale, E. S., & Tucker, C. M. (1996). Immunoassays (ELISA) for Rapid, Quantitative Analysis in the Food-Processing Industry. *Journal of Agricultural and Food Chemistry*, *44*(9), 2680–2685.

Thakur, M. S., & Ragavan, K. V. (2013). Biosensors in food processing. *Journal of Food Science and Technology*, *50*(4), 625–641.

Daniel. T. M., (2006). The history of tuberculosis. *Respirotary Medicine*, *100*, 1862–1870. https://doi.org/10.1016/j.rmed.2006.08.006

Thompson, M. (2010). Immunoanalysis- Part 2: Basic principles of ELISA. *Royal Society of Chemistry*, 1–2.

Tiberi, S., Scardigli, A., Centis, R., Ambrosio, L. D., Spanevello, A., Miguel, A., Caminero, J. A. (2017). Classifying new anti-tuberculosis drugs : rationale and future perspectives. *International Journal of Infectious Diseases*, *56*, 181–184.

Todar, K. (2012). Kenneth Todar. Retrieved November 16, 2017, from http://textbookofbacteriology.net/tuberculosis.html

Tokel, O., Inci, F., & Demirci, U. (2014). Advances in Plasmonic Technologies for Point of Care Applications. *Chemical Reviews*, *114*(11), 5728–5752.

Tran, D. P., Wolfrum, B., Stockmann, R., Pai, J.-H., Pourhassan-Moghaddam, M., Offenhäusser, A., & Thierry, B. (2015). Complementary Metal Oxide Semiconductor Compatible Silicon Nanowires-on-a-Chip: Fabrication and Preclinical Validation for the Detection of a Cancer Prognostic Protein

Marker in Serum. *Analytical Chemistry*, *87*(3), 1662–1668.

Tsai, K. S., Chang, H. L., Chien, S. T., Chen, K. L., Chen, K. H., Mai, M. H., & Chen, K. T. (2013). Childhood tuberculosis: Epidemiology, diagnosis, treatment, and vaccination. *Pediatrics and Neonatology*, *54*(5), 295–302.

Tufa, L. T., Oh, S., Tran, V. T., Kim, J., Jeong, K.-J., Park, T. J., Lee, J. (2018). Electrochemical immunosensor using nanotriplex of graphene quantum dots, Fe3O4, and Ag nanoparticles for tuberculosis. *Electrochimica Acta*, *290*, 369–377.

Van Der Merwe, P. A. (2001). Surface Plasmon Resonance. *Protein-Ligand Interactions: Hydrodynamics and Calorimetry (Practical Approach Series)*, *1*, 137–170.

van Pinxteren, L. A., Ravn, P., Agger, E. M., Pollock, J., Andersen, P. (2000). Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP10. *Clinical and diagnostic laboratory immunology,* 7(2), 155-160.

Vargas-Bernal, R., Rodrguez-Miranda, E., & Herrera-Prez, G. (2012). Evolution and Expectations of Enzymatic Biosensors for Pesticides. In *Pesticides - Advances in Chemical and Botanical Pesticides* (First, pp. 331–356). InTech.

Vashist, S. K., Schneider, E. M., Lam, E., Hrapovic, S., & H.T. Luong, J. (2014). A highly-sensitive sandwich ELISA procedure based on one-step antibody immobilization for potential _in vitro_ diagnostics. *Protocol Exchange*, 1–11.

Vetrano, A. M., Heck, D. E., Mariano, T. M., Mishin, V., Laskin, D. L., & Laskin, J. D. (2005). Characterization of the Oxidase Activity in Mammalian Catalase. *The Journal of Biological Chemistry*, *280*(42), 35372–35381.

Villemagne, B., Crauste, C., & Flipo, M. (2012). European Journal of Medicinal Chemistry Tuberculosis : The drug development pipeline at a glance. *European Journal of Medicinal Chemistry*, *51*, 1–16.

Vinuelas-Bayon, J. & Vitoria, M. A. S. S. (2017). Rapid diagnosis of tuberculosis. Detection of drug resistance mechanisms. *Enferm Infec Microbiol Clin.*, *35*(8), 518–526.

Wang, C., Knudsen, B., & Zhang, X. (2011). Semiconductor Quantum Dots for Electrochemical Biosensors.

Wang, J., Rivas, G., Cai, X., Dontha, N., Shiraishi, H., Luo, D., & Valera, F. S. (1997). Sequence-specific electrochemical biosensing of M. tuberculosis DNA. *Analytica Chimica Acta*, *337*(1), 41–48.

Wang, Q., Ju, H., Li, Y., Jing, Z., Guo, L., Zhao, Y., Wang, J. (2014). Development and evaluation of a competitive ELISA using a monoclonal antibody for antibody detection after goose parvovirus virus-like particles (VLPs) and vaccine immunization in goose sera. *Journal of Virological*

Methods, *209*, 69–75.

Wang, X. (2017). Fabrication of Electrochemical Immunosensor for Interferon-γ Determination and Its Application of Tuberculosis Diagnosis. *International Journal of Electrochemical Science*, *12*(April 1993), 7262–7271.

Wang, Y., & Bi, C. (2013). Simultaneous electrochemical determination of ascorbic acid , dopamine and uric acid using poly (tyrosine)/ functionalized multi-walled carbon nanotubes composite fi lm modi fi ed electrode. *Journal of Molecular Liquids*, *177*, 26–31.

Welin, A., Björnsdottir, H., Winther, M., Christenson, K., Oprea, T., Karlsson, A., Dahlgren, C. (2015). CFP-10 from Mycobacterium tuberculosis Selectively Activates Human Neutrophils through a Pertussis Toxin-Sensitive Chemotactic Receptor. *Infection and Immunity*, *83*(1), 205–213.

Weng, X., Gaur, G., & Neethirajan, S. (2016). Rapid Detection of Food Allergens by Microfluidics ELISA-Based Optical Sensor. *Biosensors*, *6*(2), 24.

Werner, M. T., Fæste, C. K., Levsen, A., & Egaas, E. (2011). A quantitative sandwich ELISA for the detection of Anisakis simplex protein in seafood. *European Food Research and Technology*, *232*(1), 157–166.

WHO, (2014). Guidance for National Tuberculosis Programmes on the management of tuberculosis in children - an update. In S. M. Graham (Ed.), *Treatment of TB in children.* (2nd editio, Vol. 19, pp. 82–86).

WHO, (2017). Global Tuberculosis Report 2017. Retrieved February 10, 2019, from https://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf

William, T., Parameswaran, U., Lee, W. K., Yeo, T. W., Anstey, N. M., & Ralph, A. P. (2015). Pulmonary tuberculosis in outpatients in Sabah , Malaysia : advanced disease but low incidence of HIV co-infection, *15*(32), 1–9.

Wong, Y. Y., Ng, S. P., Ng, M. H., Si, S. H., Yao, S. Z., & Fung, Y. S. (2002). Immunosensor for the differentiation and detection of Salmonella species based on a quartz crystal microbalance. *Biosensors and Bioelectronics*, *17*(8), 676–684.

Wu, J., Fu, Z., Yan, F., & Ju, H. (2007). Biomedical and clinical applications of immunoassays and immunosensors for tumor markers. *Trends in Analytical Chemistry*, *26*(7), 679–688.

Wu, L., & Qu, X. (2015). Cancer biomarker detection: recent achievements and challenges. *Chem. Soc. Rev.*, *44*(10), 2963–2997.

Wu, X., Yang, Y., Zhang, J., Li, B., Liang, Y., Zhang, C., He, J. (2010). Humoral immune responses against the Mycobacterium tuberculosis 38-kilodalton, MTB48, and CFP-10/ESAT-6 antigens in tuberculosis. *Clinical and Vaccine Immunology*, *17*(3), 372–375.

Xianga, H., Wang, Y., Wanga, M., Shaoa, Y., Jiaoa, Y., & Zhu, Y. (2013). Nanoscale. *Royal Society of Chemistry*, 1–9.

Xu, J. N., Chen, J. P., & Chen, D. L. (2012). Serodiagnosis efficacy and immunogenicity of the fusion protein of Mycobacterium tuberculosis composed of the 10-kilodalton culture filtrate protein, ESAT-6, and the extracellular domain fragment of PPE68. *Clinical and Vaccine Immunology*, *19*(4), 536–544.

Xuan, Z., Li, M., Rong, P., Wang, W., Li, Y., & Liu, D. (2016). Plasmonic ELISA based on the controlled growth of silver nanoparticles. *Nanoscale*, *8*(39), 17271–17277.

Yamamoto, M., Ushio, R., Watanabe, H., Tachibana, T., Tanaka, M., Yokose, T., Kaneko, T. (2017). Detection of Mycobacterium tuberculosis derived DNA in circulating cell free DNA from a patient with disseminated infection with digital PCR. *International Journal of Infectious Diseases*, 1–12.

Yan, R., Lynn, N. S., Kingry, L. C., Yi, Z., Slayden, R. A., Dandy, D. S., & Lear, K. L. (2011). Waveguide biosensor with integrated detector array for tuberculosis testing. *Applied Physics Letters*, *98*(1), 13702. https://doi.org/10.1063/1.3520142

Yao, C., Yu, S., Li, X., Wu, Z., Liang, J., Fu, Q., Tang, Y. (2017). A plasmonic ELISA for the naked-eye detection of chromium ions in water samples. *Analytical and Bioanalytical Chemistry*, *409*(4), 1093–1100.

Yu, Y., Zhang, X., Zhao, B., Sun, Y., Zhang, X., Bai, T., Qin, K. (2017). A sandwich ELISA for the detection of neuraminidase of avian influenza A(H7N9) virus. *Journal of Virological Methods*, *247*(April), 58–60.

Yurtsever, G., Weiss, N., Kalkman, J., van Leeuwen, T. G., & Baets, R. (2014). Ultra-compact silicon photonic integrated interferometer for swept-source optical coherence tomography. *Opt. Lett.*, *39*(17), 5228–5231.

Zajacova, Z., Hianik, T., Ostatna, V., Stoikova, E., & Evtugyn, G. (2005). Detection of aptamer – protein interactions using QCM and electrochemical indicator methods, *15*, 291–295.

Zargar, B., & Hatamie, A. (2013). Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy Localized surface plasmon resonance of gold nanoparticles as colorimetric probes for determination of Isoniazid in pharmacological formulation. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *106*, 185–189.

Zayats, M., & Willner, I. (2008). Photoelectrochemical and Optical Applications of Semiconductor Quantum Dots for Bioanalysis. *Adv Biochem Engin/Biotechnol*, *109*, 255–283.

Zhan, L., Wu, W. B., Yang, L., & Huang, C. Z. (2017). Sensitive detection of respiratory syncytial virus based on a dual signal amplified plasmonic enzyme-linked immunosorbent assay. *Analytica Chimica Acta*, *962*, 73–

79.

Zhang, Q., Prabhu, A., San, A., Al-Sharab, J. F., & Levon, K. (2015). A polyaniline based ultrasensitive potentiometric immunosensor for cardiac troponin complex detection. *Biosensors and Bioelectronics*, *72*, 100–106.

Zhang, Z., Zhu, N., Dong, S., Huang, M., Yang, L., Wu, X., Zou, Y. (2018). Plasmonic ELISA Based on Nanospherical Brush-Induced Signal Amplification for the Ultrasensitive Naked-Eye Simultaneous Detection of the Typical Tetrabromobisphenol A Derivative and Byproduct. *Journal of Agricultural and Food Chemistry*, *66*(11), 2996–3002.

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*, 1–8.

Zhu, L., He, J., Cao, X., Huang, K., Luo, Y., & Xu, W. (2016). Development of a double-antibody sandwich ELISA for rapid detection of Bacillus Cereus in food. *Scientific Reports*, *6*, 1–10.

BIODATA OF STUDENT

Noremylia Mohd Bakhori was born in Pasir Mas, Kelantan on 16th January 1987. She obtained her first degree (Bachelor of Science Honour) Chemistry in 2009 from Universiti Putra Malaysia. In 2010, she pursued her Master of Science (M. Sc) degree in Analytical Chemistry, Universiti Putra Malaysia and graduated in the year 2013. Later in 2014 she pursued her PhD degree in Sensor Technology with Institute of Advance Technology, Universiti Putra Malaysia under supervision of Prof Dr Nor Azah Yusof.

LIST OF PUBLICATIONS

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, Rahman, S.K.A., Rahman, S. F.A. (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).

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, H., Noor, S. S. M., Raston, N. H. A. and Faruq, M. (2018). Immuno Nanosensor for the Ultrasensitive Naked Eye Detection of Tuberculosis. *Sensors*, 18(6), 1932.

AbuHassan, K., **Bakhori, N. M.,** Kusnin, N., Azmi, U.Z.M, Tania, M.H., Evans, B., Yusof, N.A., and Hossain, M. A. (2017). Automatic Diagnosis of Tuberculosis Disease Based on Plasmonic ELISA and Color-based Image Classification. *39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*.

Tania, M.H., Lwin, K. T., AbuHassan, K., **Bakhori, N.M**., MohdAzmi, U.Z., Yusof, N.A. and Hossain, M. A. (2017). An Automated Colourimetric Test by Computational Chromaticity Analysis: A case study of Tuberculosis Test. *11th International Conference on Practical Applications of Computational Biology & Bioinformatics (PACBB'17)*, Polytechnic of Porto - Porto (Portugal), 21-23 June 2017.

List of Presentations

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, H., Immunonanosensor for ultrasensitive and affordable naked eye detection of tuberculosis. In 29th Malaysian Analytical Chemistry Symposium, 15th -17th August 2016, Bayview Beach Resort, Penang, Malaysia.

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, H., Immunonanosensor for ultrasensitive and affordable naked eye detection of tuberculosis. In Workshop on Advanced Materials and Technology 2015 (WAMN 2015), 4th – 5th November 2015, Auditorium Faculty of Engineering, UPM.

Bakhori, N. M., Yusof, N. A., Abdullah, J., Wasoh, H., Immunonanosensor for ultrasensitive and affordable naked eye detection of tuberculosis. Fundamental Science Congress (FSC 2015), 12th – 13th November 2015, Faculty of Veterinar, UPM.

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