

An assessment of two evanescent field biosensors in the development of an immunoassay for tuberculosis

by

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I declare that the thesis/dissertation, which I hereby submit for the degree PhD (Biochemistry) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE : ##

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"I can do all things through Christ who strengthens me".

SUMMARY

Title: An assessment of two evanescent field biosensors in the development

of an immunoassay for tuberculosis

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Accurate diagnosis of active tuberculosis is required to improve treatment, reduce transmission of the disease and control the emergence of drug resistance. A rapid and reliable test would make a considerable contribution to the management of the TB epidemic, especially in HIV-burdened and resource-poor countries where access to diagnostic laboratories are limited. Surrogate marker antibody detection to mycobacterial lipid cell wall antigens gave promising results, in particular with cord factor. The specific advantage of using mycolic acids as lipid antigens in comparison to protein antigens is that mycolic acid is a CD1 restricted antigen with the ability to induce proliferation of CD4/CD8 double negative T-cells, which may explain the sustained antibody production in AIDS patients. Traditional end-point assays to detect anti-MA antibodies showed an unacceptable number of false positive and negative test results. Here a much improved biosensor method (the MARTI-assay, i.e. Mycolic acid Antibody Real-Time Inhibition assay) was developed to detect antibodies to mycolic acid in patient sera as surrogate markers of active tuberculosis. The test was assessed on an IAsys optical biosensor and gave an accuracy of 82%. The technology was transferred to an SPR (ESPRIT) biosensor to economise and simplify the assay. Mycolic acid containing liposomes were immobilized on the SPR gold surface precoated with octadecanethiol. The following parameters were optimized on the ESPRIT biosensor to enable reliable TB diagnosis: effect of degassed buffer, saponin blocking, first exposure to serum at low concentration and second exposure to antigen inhibited serum at high concentration. The IAsys biosensor system has a weakness in the double channel cuvette system, in which the channels often do not give matching results, while being ten times more expensive than the gold discs provided for the



ESPRIT biosensor. The ESPRIT biosensor is provided with an adjustable laser setting to compensate for differences in the channel readings as well as an automated fluidic system that reduces variance from one sample to the next. First indications are that the test can also be used for prognosis of TB during treatment. It is hoped that the ESPRIT biosensor will improve the accuracy of the test to more than 90%. If the MARTI-assay technology could be made amenable for high throughput screening, it may provide the solution to the serodiagnosis of tuberculosis and monitoring of progress during TB treatment both in adult and children, thereby reducing the spread of TB within the communities.



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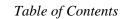
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List of Abbreviations

AFB Acid-fast bacilli

AFM Atomic force microscopy

AG Arabinogalactan

AIDS Acquired immune deficiency syndrome

APC Antigen presenting cell
ART Antiretroviral therapy

ARV Antiretroviral

ASI Artificial sensing instrument

Au Gold

BCG Bacillus Calmette-Guerin

CMD Carboxymethyl dextran

CO₂ Carbon dioxide

CPC Cetyl pyridinium chloride

CFP Culture filtrate protein

CIC Circulating immune complex

CV Cyclic voltammetry

DAT 2,3-diacyl trehalose

DIBA Dot immunobinding assayDNA Deoxyribonucleic acid

ESAT Early secretory antigenic target

EDC Ethyl-dimethylaminopropyl carbodiimide

EDCTP European and Developing Countries Clinical Trials

Partnership

EDTA Ethylene diamine tetra-acetic acid

ELISA Enzyme linked immunosorbent assay

ELISPOT Enzyme-linked immunospot



F Frequency

FET Field effect transistor

FIND Foundation for Innovative New Diagnostics

hr Hour

HCl Hydrochloric acid

HDL High density lipoprotein

HIV Human immunodeficiency virus

IAsys Interaction analysis system

IDL Intermediate density lipoprotein

IgGImmunoglobulin GIFN-γInterferon gamma

INH Isoniazid

IRIS Immune reconstitution inflammatory syndrome

KCl Potassium chloride

kDa Kilodalton

KOH Potassium hydroxide

LAM Lipoarabinomannan

LAPS Light addressable potentiometric sensor

LDL Low density lipoprotein

LED Light emitting diode

LM Lipomannan

MA Mycolic acids

mAGP Mycolyl-arabinogalactan peptidoglycan

MARTI Mycolic acid Antibody Real-Time Inihibition

MDR Multi-drug resistance



MHC Major histocompatibility complex

Min Minute

MS Mass spectroscopy

M.tb Mycobacterium tuberculosis

MTBDR Mycobacterium tuberculosis drug resistance

NaCl Sodium chloride

NaOH Sodium hydroxide

neg Negative

NHS *N*-hydroxy-succinimide

NTA Nickel chelating surface

NTM Non-tuberculosis mycobacteria

ODT Octadecanethiol

PBS/AE Phosphate buffered saline azide EDTA

PCR Polymerase chain reaction

PEG Polyethylene glycol

PGL Glycolipid

PIM Phosphatidyl inositol mannosides

pos Positive

PPD Purified protein derivative

QCM Quartz crystal microbalance

RIfS Reflectometric interference spectroscopy

RNA Ribonucleic acids

rRNA Ribosomal ribonucleic acids

RU Resonance units

SAM Self-assembled monolayer



SDS Sodium dodecylsulphate
SEM Standard error of the mean

SPR Surface plasmon resonance

TAT 2,3,6-triacyl trehalose

TB Tuberculosis

TDM Trehalose dimycolate

TIR Total internal reflection

TMM Trehalose monomycolate

TST Tuberculin skin test

VLDL Very low-density lipoprotein

WHO World Health Organization

XDR Extensively drug-resistant



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