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

by

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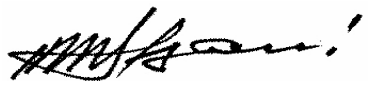
SIGNATURE : 

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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 pre-coated 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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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
CO_2	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 assay
DNA	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
IgG	Immunoglobulin G
IFN- γ	Interferon 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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