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Evaluation of a T-cell Assay for *Mycobacterium tuberculosis* Infection in The Gambia



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Abstract

New generation T cell assays offer hope in the diagnosis of *Mycobacterium tuberculosis* infection and disease. We assessed the ELISPOT assay using cross-sectional and longitudinal studies and a natural gradient of *M. tuberculosis* exposure by sleeping proximity to a tuberculosis (TB) case in The Gambia. Two antigens, ESAT-6 and CFP-10 (EC), were compared to purified protein derivative (PPD) by ELISPOT and to the PPD skin test in 735 TB contacts. All three tests responded to the exposure gradient, the PPD skin test most dramatically. Inter-test comparison showed that the EC ELISPOT provided improved specificity in the diagnosis of *M. tuberculosis* infection, but at the cost of some sensitivity. Increasing discordance, particularly between PPD ELISPOT and PPD skin test results, down the exposure gradient to 105 community controls was identified. In 693 children, the EC ELISPOT was slightly less sensitive than the PPD skin test in the diagnosis of *M. tuberculosis* infection from recent exposure; neither test was confounded by prior BCG vaccination, even in the very young. A fusion protein of EC compared favourably with their respective peptides by ELISPOT assay in 488 TB contacts, a combined test result offered improved sensitivity. Quantitative ELISPOT and PPD-skin test responses were assessed in 1052 TB case contacts, according to an ELISPOT response to EC. Only the ELISPOT count was sensitive to the exposure gradient ($p=0.009$), revealing a positive dose-response relationship. In the longitudinal assessment, both ELISPOT and PPD skin test conversion occurred over time. PPD skin test reversion occurred in 10% of individuals after 18 months, ELISPOT reversion occurred in 39% at 3 months. In conclusion: the EC ELISPOT offers increased specificity in the diagnosis of *M. tuberculosis* infection in The Gambia, at the cost of some sensitivity; the PPD skin test appears to be down-regulated in the community; neither test is confounded by prior BCG vaccination; a fusion protein in combination with EC peptides offers optimal ELISPOT sensitivity; the quantitative ELISPOT response in specific-antigen-positive TB case contacts reflects the infectious load of *M. tuberculosis*; and significant early reversion of the ELISPOT test suggests it is unreliable in *M. tuberculosis* dormancy.

I dedicate this thesis to Marian, my wife and best friend, who established our home in The Gambia and 'hung in there' so amazingly well. I could not have done this without you.

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Name and role of each person who contributed to the studies of this thesis, to the level of 'author' status. Names are listed in alphabetical order of the surnames.

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Roger Brookes	Immunologist. Joint coordinator of the ELISPOT work, involved in analysis and write up of all the studies.
Tumani Corrah	Clinician-scientist – head of clinical services, MRC Gambia. Involved in the clinical care of the TB cases and contacts.
Simon Donkor	Data manager. Involved in design of the databases for all the studies. Supervised data collection and verification.
Katherine Fielding	Statistician, London School of Hygiene and Tropical Medicine. Involved in the design of the case contact work in The Gambia. Led the statistical analysis of chapter 2, and involved in the write up of that study.
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Abbreviations

BCG	Bacille Calmette-Guérin
C	Centigrade
CI	Confidence Interval
E	Eastern
EC	ESAT-6/CFP-10
ELISPOT	Enzyme Linked Immunospot assay
IFN- γ	Interferon gamma
kDA	kilo-Dalton
LAL	Limulus Amebocyte Lysate
L-J	Lowenstein-Jensen
MRC	Medical Research Council
n	Number
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PHA	Phytohaemagglutinin
PPD	Purified Protein Derivative
RD	Region of Difference
rt-PCR	Reverse transcriptase Polymerase Chain Reaction
SFU	Spot Forming Units
SSI	Statins Serum 'Institut'
TB	Tuberculosis
TH	T Helper cell
TST	Tuberculin Skin Test
TU	Test Units
UK	United Kingdom
WHO	World Health Organisation
ZN	Ziehl-Neelsen

Articles arising from these studies

1. Hill P.C, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, Lugos M, Owiafe PK, Donkor SA, Hammond AS, Otu JK, Corrah T, Adegbola RA, McAdam KPWJ. Large-Scale Evaluation of Enzyme-Linked Immunospot Assay and Skin Test for Diagnosis of *Mycobacterium tuberculosis* Infection against a Gradient of Exposure in The Gambia. Clin Infect Dis 2004 ; 38 :966-73.¹
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3. Hill PC, Fox A, Jeffries DJ, Jackson-Sillah D, Lugos MD, Owiafe PK, Donkor SA, Hammond AS, Corrah T, Adegbola RA, McAdam KPWJ, Brookes RH. Quantitative T cell assay reflects infectious load of *Mycobacterium tuberculosis* in an endemic case contact model. Clin Infect Dis 2005; 40:273-8.³
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7. Hill PC, Fox A, Jackson-Sillah D, Jeffries DJ, Lugos MD, Adegbola RA, McAdam KP, Brookes RH. Longitudinal assessment of the ELISPOT assay for *Mycobacterium tuberculosis* infection. (In preparation).