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2005

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A thesis in fulfilment of the requirements for the degree of Doctor of Medicine,
the University of Auckland, 2005.
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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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


