

# Utility of the ELISPOT Assay in the Diagnosis of Latent Mycobacterium tuberculosis Infection Among HIV-Infected Patients With Advanced Immunodeficiency in South Africa



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## UPDATED ABSTRACT

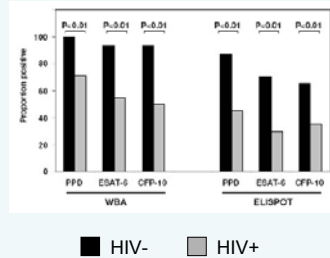
**BACKGROUND:** ELISPOT assays incorporating *Mycobacterium tuberculosis*-specific antigens (ESAT-6 and CFP-10) are useful in the diagnosis of tuberculosis (TB) and latent infection and may reflect mycobacterial burden. However, determinants of ELISPOT IFN-γ responses to these antigens have not been assessed in HIV+ patients with advanced immunodeficiency living in settings with high TB burden.

**METHODS:** Tuberculin skin tests (TSTs), overnight IFN-γ ELISPOT and 7-day whole blood assays (WBA) incorporating ESAT-6 and CFP-10 were compared in HIV+ (n=40) and healthy HIV- (n=30) controls without active TB in a community with very high tuberculosis (TB) incidence.

**RESULTS:** TSTs, ELISPOTs and WBAs were each positive in ≥70% of HIV- controls. These tests were positive in significantly lower proportions of HIV+ individuals (median CD4 count=114 cells/μl) (P<0.01 for each comparison). TSTs and WBA quantitative responses (but not ELISPOTs) were significantly abrogated in patients with CD4 cell counts <100 cells/μl. 19 HIV+ patients had recently completed treatment for TB and positive ELISPOT responses were far less frequent in those who had received treatment than those who had not (11% versus 62%, P<0.001). In multivariate analysis, ELISPOT responses (but not TST or WBA) showed a strong inverse association with a history of recent TB treatment (adjusted OR=0.06, 95%CI=0.10-0.40, P<0.01) but were independent of CD4 count and viral load. Among HIV+ individuals who had not received TB treatment both the magnitude and proportion of positive ELISPOTs (but not TST or WBA) were similar to those of HIV-negative controls.

**CONCLUSIONS:** ELISPOT responses in HIV+ patients with advanced disease were independent of CD4 cell count but had a strong inverse association with history of TB treatment (reflecting likely mycobacterial clearance). ELISPOT assays are likely to be useful for patient assessment and as an immuno-epidemiological tool in high HIV prevalence settings.

## COMPARISON IFN-γ ELISPOT AND IFN-γ WHOLE BLOOD ASSAY (WBA)



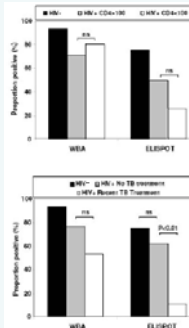
In initial analyses, both WBA and ELISPOT assays appeared to be impaired in those with HIV infection

## SETTING

Guguletu- Nyanga District, Cape Town, South Africa  
Population: 419 000  
HIV prevalence : 28.5%  
TB incidence approx. 1,400 / 100,000 / yr



## FACTORS ASSOCIATED WITH POSITIVE IFN-γ ASSAYS IN HIV+ GROUP



Using the ELISPOT assay, both the proportion of positive responses and the magnitude of responses

- a) were not significantly associated with low CD4 cell count
- b) were very strongly associated with recent completion of successful TB treatment

ELISPOT responses among HIV+ patients with no history of TB treatment had similar ELISPOT responses to the HIV- group

## Patients

HIV+ patients without TB enrolling into an ART clinic (n=40)

- Median age 31 years
- Median CD4= 114 cells/ul
- 48% had a history of completed TB treatment

Healthy HIV- controls (n=30)

- Median age 28 years
- 0% had previously had TB

## ASSAYS

1. TST 2TU RT23 PPD
2. ELISPOT
  - Overnight IFN- γ assay
  - 2.5x10<sup>6</sup> cells/well
  - Recombinant ESAT-6 / CFP-10
  - Cut-off ≥20 SFU / 10<sup>6</sup> PBMC
3. Whole blood assay (WBA)
  - diluted whole blood
  - Antigen stimulated for 7 days
  - IFN-γ measured in supernatants

## MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH A POSITIVE ELISPOT TO ESAT-6 / CFP-10 IN HIV+ PATIENTS

Variable	Crude OR	P value	Adjusted OR	P value
Age	<31 years	1.00	-	-
	>31 years	0.58 (0.16-2.12)	0.41	-
Sex	Male	1.00	-	-
	Female	5.44 (0.60-49.56)	0.13	-
CD4 cell count (cells/μl)	>100	1.00	1.00	-
	<100	0.34 (0.08-1.34)	0.12	0.58 (0.11-3.16)
Log viral load (copies/ml)	<5.0	1.00	-	-
	>5.0	0.39 (0.10-1.49)	0.17	0.30 (0.05-1.69)
History of TB	No	1.00	-	-
	Yes	0.07 (0.01-0.40)	<0.01	0.06 (0.10-0.40)

## TST Results



Induration ≥10 mm seen in: 42% of HIV+ patients versus 77% of HIV- patients (P<0.05)

- TST responses in HIV+ individuals associated with
- CD4 count <100 cells/ul OR=0.14 (P=0.03)
  - Viral load >5 log copies/ml OR=0.18 (P=0.04)

## CONCLUSIONS

Approximately ~70-80% of HIV-neg people have latent MTB infection in this community

TSTs were anergic in a high proportion of HIV+ patients and were associated with low CD4 cell count and high viral load

Overnight ELISPOT responses were

- independent of CD4 cell count
- were only associated with history of TB treatment (probably reflecting mycobacterial clearance)

Overnight ELISPOTs (but not TST or WBA) are likely to be useful for patient assessment for MTB infection and as an immuno-epidemiological tool in high TB and HIV prevalence settings.