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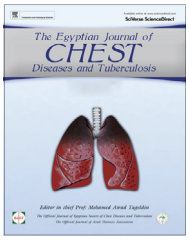

ORIGINAL ARTICLE

# ESAT-6-ELISpot and interferon γ in the diagnosis of pleural tuberculosis

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**Abstract** *Background:* Appropriate diagnostic methods for tuberculous pleural effusion are vital. The IFN-γ tests using specific *Mycobacterium Tuberculos* is antigens in samples from the site of infection may be promising in diagnosis of tuberculosis. Objective we examined the ability of ELISpot test using circulating peripheral blood mononuclear cells (PBMC) and compartmentalized pleural fluid mononuclear cells (PFMC) for diagnosis of active TB infection in patients with tuberculous pleural effusion. Methods PBMC and PFMC-based ELISpot test for IFN-γ test using specific *M. tuberculosis* antigen: Early Secretory Antigen Target-6 protein (ESAT-6) was used for diagnosis of active TB infection. Thirty-five patients with clinically suspected tuberculous pleural effusion were enrolled over a 12-month period. Results 11 patients out of 35 were positive by culture and PCR (31.4%). Incubation of PBMC with ESAT-6 for 8 h showed sensitivity and specificity of 82% and 92%, respectively, for the PBMC–ELISpot as compared to PFMC–ELISpot that was 54% and 96% respectively. With 24 h incubation of ESAT-6 there was around 2.5 fold increase in the median number of spot forming cells (SFCs) in PFMC from 30 to 74, whereas there was minimal increase of median number of SFCs in PBMC from 55 to 60. Conclusion ESAT-6 – ELISpot using PBMC and PFMC is useful as a tool for diagnosis of TB effusion. PFMC needs longer period of incubation for processing of ESAT-6 than PBMC. Moreover, IFN-γ in pleural effusion (PE) is another useful way for diagnosis of TB pleurisy which is sensitive, simple and cheap.


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## Introduction

Tuberculosis (TB) is a major public health problem and it is estimated by the World Health Organization that there are approximately 9.4 million incident cases, and 1.3 million deaths among HIV-negative people [1]. The disease is especially prevalent in developing countries, where it accounts for

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more than a quarter of all preventable adult deaths [2]. The diagnosis of tuberculous pleural effusion remains a serious clinical problem. Routine analysis of pleural fluid, signs and symptoms and radiological finding are often inadequate for starting of empirical therapy.

Currently, the only sure criterion for definite diagnosis of TB is the demonstration of the presence of tubercle bacilli in clinical specimens. This is based on traditional methods: the Ziehl-Neelsen (ZN) acid fast stain and laboratory culture of *Mycobacterium tuberculosis* on Lowenstein Jensen media (LJ) medium. However, ZN stains lacks specificity and sensitivity, whilst confirmation by culture requires several weeks [3]. Although, new rapid diagnostic culture methods have been investigated that are based on either bacteriophage detection methods [4] or liquid culture technique such as BACTEC [5], they require specialized personnel and equipment. In addition their expenses have limited their use in many routine diagnostic laboratories.

Several newly developed diagnostic assays for TB based on *M. tuberculosis* – specific antigens encoded by genes in the region of difference 1 (RD1 region) gave promising results for diagnosis of *M. tuberculosis* infection. These specific antigens such as early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are proved to be accurate marker of *M. tuberculosis* infection and to distinguish *M. tuberculosis* infection from the response to BCG vaccination [6].

ESAT-6 and CFP-10 forms 1:1 complex when co expressed in an engineered BCG strains which found to be difficult to be processed and presented to T Cells. Consequently, single antigen is preferred to be used rather than ESAT-6 and CFP-10 form [7]. ESAT-6 or CFP-10 have been frequently used in Enzyme-linked immunospot (ELISpot) [8–10], Flow-cytometry [7,11–14] and Quantiferon assays [15,16]. The usefulness of these assays for diagnosis of tuberculous pleural effusion in actual clinical practice is limited especially in endemic area. It has been shown that mononuclear cells (MC) compartmentalized in the infected sites such as pleural fluid [17], bronchoalveolar lavage fluid [18] and CSF [12,19] secrets higher IFN-γ in infected sites than peripheral blood mononuclear cells.

In this study we aimed to evaluate the ability of ELISpot test using circulating peripheral blood mononuclear cells (PBMC) and compartmentalized pleural fluid mononuclear cells (PFMC) for diagnosis of active TB infection in patients with tuberculous pleural effusion.

## Materials and methods

This study was done at Chest and Microbiology & Immunology Departments, Zagazig University Hospitals between November 2009 and October 2010. Written informed consents were taken from all subjects.

Thirty-five patients with suspected TB pleural effusion (ages 37 ± 11.2 years, male/female = 24/11) were included. The following were done:

(1) Full clinical examination.
(2) Plain chest X ray and chest CT
(3) Pleural fluid tapping was performed according to the standards procedures (20). Cytological, microbiological ZN stain, culture, and pleural fluid PCR were processed using standard techniques [8,12].
(4) Thoracoscopy and/or Abram's pleural biopsy was performed to cases in which final diagnosis was not reached by the previous procedures.
(5) A total of nineteen patients with non-tuberculous pleural effusion were enrolled as a control in this study (ages 32 ± 7 years, male/female = 15/4).

### *Antigen*

ESAT-6 was produced as recombinant proteins in *Escherichia* coli (13).

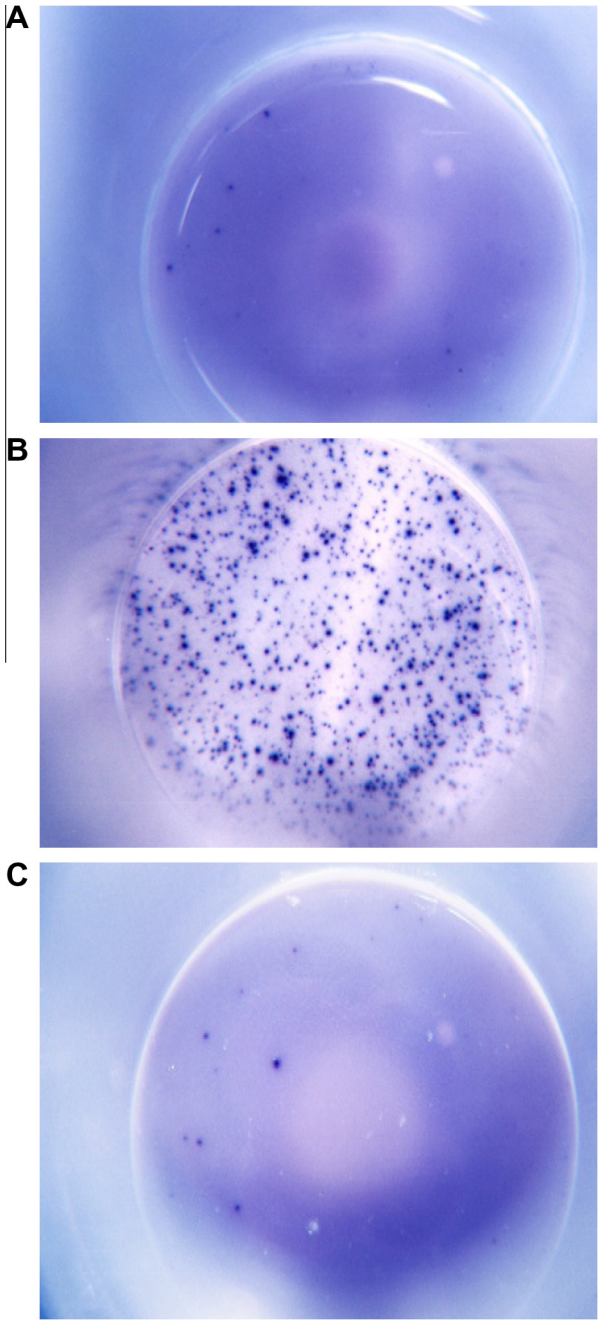


**Figure 1** IFN-γ producing T-cell responses (A) (Negative control), (B) (Positive Control) and (C) (ESAT-6).