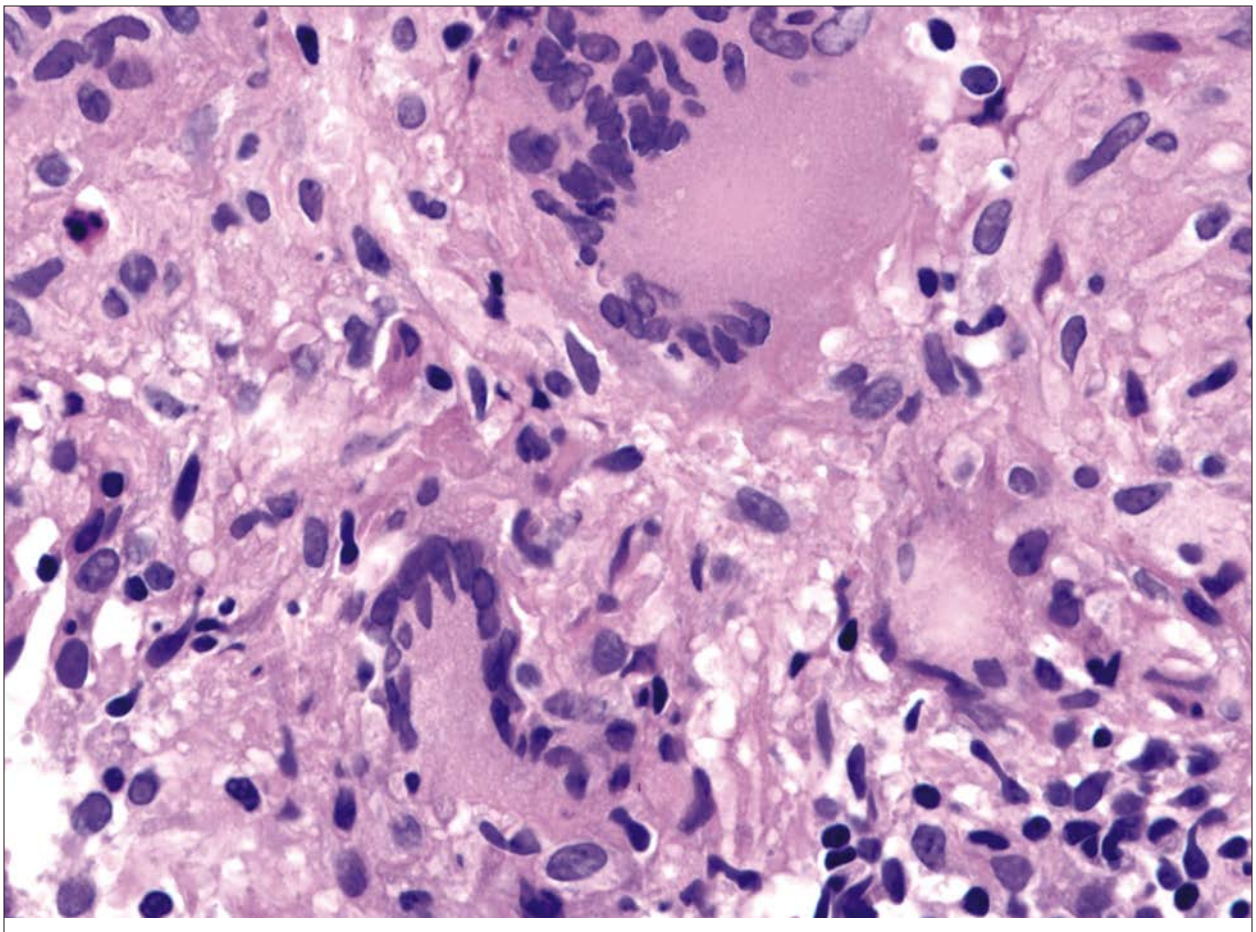

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Immunological and Molecular Diagnosis of *Mycobacterium tuberculosis* Between two Environmentally Different Regions

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Abstract: *Mycobacterium tuberculosis* (*M. tuberculosis*) is an airborne contagious disease that is transmitted by coughing, sneezing or even talking. Once a person becomes infected, any condition that weakens the immune system can trigger the development of active *M. tuberculosis*. Exposure to smoke can increase the risk of *M. tuberculosis* by reducing resistance to initial infection or by promoting the development of active *M. tuberculosis* in already infected persons. Exposure to the pollutants contained in biomass smoke or other sources of air pollution can weaken the immune system, impair the lungs and make them more susceptible to infection and disease. The association between air pollution and respiratory infections is well understood but the relation between air pollution and tuberculosis is not. So, it is anticipated that the prevalence of *M. tuberculosis* is positively correlated with environmentally polluted areas. In order to compare the prevalence of *M. tuberculosis* in two different areas in Alexandria city, industrial area (showing petroleum oil, fertilizer and cement industries) and residential area, golden standard methods [acid fast staining and culture] and molecular assays [Polymerase Chain Reaction technique (PCR) and Enzyme-Linked Immunospot assay (ELISpot)] of *M. tuberculosis* detection were used. There was a significant increase in the prevalence of *M. tuberculosis* in the industrial district than the residential one. Molecular tool of *M. tuberculosis* diagnosis was found to be the best than immunological method. Air pollution produced by petroleum, fertilizer and cement industries most likely has major effect on increasing the prevalence of *M. tuberculosis* infection. On the other hand, the power of the molecular techniques was confirmed and showed to be more sensitive than the traditional methods and the PCR assay was more accurate than the ELISpot assay.

Key words: *M. tuberculosis*, air pollution, industry pollution, diagnosis, PCR, ELISpot

INTRODUCTION

It is well characterized that mammalian host defense against *M. tuberculosis* depends on two types of immunity: innate and adaptive (Kaufmann, 2001). On the other hand, several investigators have reported that industrial pollution in air (out and indoor), water and heavy metals can badly affect human and animals immune defense against infections (Chalupka, 2005).

Tuberculosis causes an estimated two million deaths per year (Maher and Raviglione, 2005), the overwhelming majorities occur in developing countries (Raviglione *et al*., 1995) and it infects approximately one third of the world's population (Dye *et al*., 2005). The incidence of *M. tuberculosis* has stabilized or is on the decrease in many parts of the world, While the incidence rate is rising by approximately 350 cases per 100 1000 population in Africa (WHO, 2007)

The conventional methods for laboratory diagnosis of *M. tuberculosis*, including acid fast staining and culturing are either insensitive (Hajime *et al*., 2002) or time-consuming (Pfyffer *et al*., 2003). For

enhancing treatment strategies, plus efficacy markers to compare them, new diagnostic tools for *M. tuberculosis*, were needed to help combat the epidemic disease, in which the use of nucleic acid amplification and detection in sputum, blood and body fluids may provide quick and specific results for identifying the *M. tuberculosis* complex (Wang *et al*., 2004; Piersimoni and Scarparo, 2003). Recent developments of immune-based assays to detect *M. tuberculosis* infection are a significant advance. ESAT-6 and CFP-10 are two proteins encoded by the RD1 genomic segment of *M. tuberculosis*, which is absent from all BCG strains and the vast majority of environmental mycobacteria. As a result, enzyme-linked immunospot (ELISPOT) assays that detect interferon-gamma (IFN-γ) release in response to these antigens differentiate between *M. tuberculosis* infection and immune sensitisation by BCG vaccination or exposure to environmental mycobacteria (Hill *et al*., 2004, 2005; Nicol *et al*., 2005). However, its performance in rapid diagnosis of active *M. tuberculosis* in disease-endemic areas is still unknown. In the present, we have two targets, the first was to compare between the different methods of Diagnosis on which the ELISpot (T SPOT- *M. tuberculosis*) assay in clinically suspected cases of *M. tuberculosis* was evaluated in comparison to the molecular detection using specific PCR system, in which blood samples were used. Both methods were carried out in comparison with the gold standard method of *M. tuberculosis* detection (acid fast staining and culturing). The second target was to compare between two environmentally different regions to study the effect of pollution on the *M. tuberculosis* infection.

MATERIALS AND METHODS

Population Study

The study was conducted in the year 2006/2007 where, the population study comprises 100 persons (suspected to be infected with *M. tuberculosis*) simply random selected from those attendants to El-Maamora hospital for chest disease in Alexandria for check up, 50 living in an industrial district matched for age and sex with 50 individuals living in residential district both districts are in Alexandria, Egypt. A written consent was taken from each person involved in this study. On enrollment, each person was interviewed and their medical records reviewed.

Blood samples were collected from each person for DNA extraction which used in the amplification by PCR and ELISpot tests.

Complete Medical History

Sex, age, residence, occupation, living conditions and personal habits such as smoking were registered. Family history of Chronic Obstructive Pulmonary Disease (C.O.P.D), bronchial asthma or *M. tuberculosis* was also recorded.

Medical Examination

Anthropometric measurement (weight, height) and calculation of body mass index [wt (kg) ht^{-1} $(m)^2$] was recorded. In addition to general medical examinations including history, symptoms, signs, radiologic, pathologic and microbiological results and follow-up observations were carefully reviewed.

Routine Laboratory Evaluation

This examination was performed for each person according to his compliant (as urine examination, stool examination and complete blood count). Sputum samples were collected for 3 consecutive days then subjected routine work examination by direct smear and culture examination.

Chest Radiography

Individuals who had cough and other complaints related to respiratory system were subjected to chest radiography.

Methods for Detection of *M. tuberculosis* Traditional Assay

Ziehl Neelsen (Zn) Stain

The standard method for direct smear examination was carried out according to Boyed (1995).

Culture Method

According to Metchock *et al*. (1999), a direct inoculum was cultured on conventional solid medium (the egg-based Lowenstein- Jensen -LJ) and incubated at 37°C for 28 days.

Molecular Assays

Total DNA Extraction of Blood Samples

Peripheral Blood Mononuclear Cell (PBMCs) were isolated from heparinized blood sample using lymphocyte separation buffer and gradient centrifugation at 2000 rpm for 30 min at room temperature (ficoll solution, density of 1.077 g mL^{-1}, Seromed, Germany). Isolated cells were washed 2X by Phosphate Buffered Saline (PBS). Each million cells were suspended in 100 µL proteinase K buffer (50 mM Tris pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, 0.45% Nonidet 40) containing 100 µg mL^{-1} proteinase K, followed by incubation at 56°C for 3 h, then at 95°C for 15 min to inactivate the enzyme, finally the DNA was purified by phenol/chloroform extraction and ethanol precipitation (Mirza *et al*., 2003).

***M. tuberculosis* Detection by PCR Method**

M. tuberculosis DNA was detected in the blood samples by amplification of IS6110 insertion sequence. It was performed using sequence specific primers in a nested PCR reaction as described (El Demellawy *et al*., 2006). Briefly, The first PCR amplification of IS6110 insertion sequence was carried out in a mixture containing DNA concentration 70-100 ng, 1X PCR buffer, 200 µM dNTPs (Amersham Biosciences, Freiberg, Germany), 1 µM of each of the external primers, the forward primer *M. tuberculosis* 1 with sequence 5`-GGA CAA CGC CGA ATT GCG AAG GGC-3` and the reverse primer *M. tuberculosis* 2 with sequence 5`-TAG GCG TCG GTG ACA AAG GCC ACG-3`, 15 mM $MgCl_2$ and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Scotland, UK). The amplification was initiated by one cycle as follows: denaturation at 95°C for 5 min, annealing at 65°C for 1 min, then extension at 72°C for 1.5 min followed by 30 cycles, each as follows: denaturation at 95°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 1.5 min A final extension at 72°C for 15 min was performed. Size of the amplified fragment after this round was 580 bp. Nested PCR amplification was carried out using 5 µL of the first PCR amplified product in a PCR mixture containing 1X PCR buffer, 200 µM dNTPs, 1 µM of each of the internal primers, the forward primer *M. tuberculosis* 3 with sequence 5`-ACG ACC ACA TCA ACC-3`and the reverse primer *M. tuberculosis* 4 with sequence 5`-AGT TTG GTC ATC AGC C-3`, 15 mM $MgCl_2$ and 1 U *Taq* DNA polymerase for 20 cycles, each consists of denaturation at 95°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1.5 min followed by extra extension at 72°C for 15 min. This step will give amplified fragment of 181 bp.

***M. tuberculosis* Detection by Elispot Assay**

ELISpot was performed by using a commercial kit (R and D System, Inc. UK) as previously described in Nicol *et al*. (2005). Briefly, 96-well polyvinylidene fluoride–backed plates were coated with 15 µg mL^{-1} of monoclonal antibody 1-D1K against IFN-γ. *M. tuberculosis* specific antigen (ESAT-6 or CFP-10) or mitogen was added followed by addition of purified PBMCs (250,000/well) in duplicate wells. No antigen was added to the background control wells. After incubation for 18 h, plates were washed, 100 µL (1 µg mL^{-1}) of biotinylated monoclonal antibody 7-B6-1-biotin against IFN-γ was added and incubated for 2 h followed by washing with streptavidin-alkaline phosphatase toxoid and incubated for 1.5 h; plates were washed again and 100 µL of chromogenic alkaline phosphatase substrate was added. After 10-15 min, the plates were washed and spots were

enumerated with a stereomicroscope independently by 2 observers. Mean values determined by the 2 observers and both duplicate wells were used in all calculations. The number of spots in the background control wells was subtracted from the number in the test wells and a response was considered positive if the number of spots per test well was >10 and at least twice the value found in the background control wells.

Statistical Analyses

The data were subjected to statistical analysis using SPSS version 11 to estimate Probability (P) value and Chi-square (χ^2), where probability will be significant when its values $p \leq 0.005$ and Chi-square (χ^2) is a statistical model used for comparison between distributions of patients according to different environmental variables of the study.

RESULTS AND DISCUSSION

The environmental parameters effects on the prevalence of *M. tuberculosis* infection was detected by analyzing data of the *M. tuberculosis* patient statistically using Chi square (χ^2) test according to different factors including age, sex, crowding index, family income, occupation, smoking and medical family history (Table 1). From this analysis it was concluded that, the major affected age is in the range of 20-30 years old in the residential area and 30-40 years old in the industrial area. Regarding the sex factor, men were more affected in industrial area than the women in which is understood due to their exposure to the industrial pollution more than female. The medical family history has great role in the *M. tuberculosis* infection, where, *M. tuberculosis* was detected clearly in the families that suffer from *M. tuberculosis* and asthma especially in the industrial area, this might be due to the fact that the chance of infection becomes optimum, when there is infectious disease within the family itself (Perez-Padilla *et al*., 2001; Thierry *et al*., 1994). On other hand, the smoking habits by either cigarettes or nargila smoking proved to be an effective reason for *M. tuberculosis* infection. The nargila smokers specially who are living in the industrial area were more susceptible to infection than the cigarette smokers which could be due to the increase in probability cross infection through the smoking apparatus. However, either methods of smoking proved to have significant influence on *M. tuberculosis* infection as shown in Table 1.

Crowded areas could be also considered as a significant factor on *M. tuberculosis* infection, especially in industrial area. This could be owing to the easily distribution of the infectious disease within a restricted area with a bad ventilation condition and overcrowded with infected people. In addition to the previous factors, the financial level has a great role in the disease handling and control. As the income of the family increase the disease control, consequently, the number of infected patients will decrease as reported here.

On studying the occupation effect on the *M. tuberculosis* infection, it was found that, the nearer to the risky areas, the more effectively *M. tuberculosis* infection occurs. This can be due to the more exposure to the most pollution sources that can affect their sensitivity and infection with *M. tuberculosis* . The current research reveled that, family history [$\chi^2 = 5.47$, $p = 0.019$] is the most effective parameter that influence the infection of *M. tuberculosis* . The incidence of *M. tuberculosis* percentage was jumped up to 46 in Industrial area, while dropped down to 16 in residential area. In other words, the incidence of *M. tuberculosis* in industrial area was more than 2.8 times the incidence of *M. tuberculosis* in residential district. This study emphasizes the important role of industrial pollution on the prevalence of the *M. tuberculosis* as we expected from our hypothesis and in agreement with previous reports by Mishra *et al*. (1999) and Gupta *et al*. (1997).

In order to make successful comparison between the prevalence of *M. tuberculosis* in industrial area and residential area, we have used traditional methods (acid fast staining and culture) and molecular diagnostic tests (PCR and ELISpot). As indicated in Table 2a and b, the overall tests sensitivity,

Table 1: Socio-demographic data of the studied two group

	Industrial areas n = 50		Residential area n = 50		χ^2
Criteria	No.	%	No.	%	p
Age					
10-20	3	6	3	6	2.53
20-30	17	34	16	32	0.63
30-40	14	28	19	38	
40-50	10	20	5	10	
>50	6	12	7	14	
Sex					
Male	30	60	29	58	0.04
Female	20	40	58	42	0.83
Crowding index					
3 room	20	40	21	42	0.04
Less 3 room	30	60	29	58	0.83
Family income					
Enough	17	34	21	42	0.70
Not enough	33	66	29	58	0.705
Occupation					
House wife	16	32	19	38	2.12
No	8	16	7	14	0.346
Risky work	11	22	8	16	
Not risky work	15	30	16	32	
Smoking					
Yes	30	60	30	60	0.40
No	11	22	9	18	0.818
Passive smoker	9	18	11	22	
Family history					
Negative	11	22.0	22	44.0	5.47
Positive	39	78.0	28	46.0	0.019*
M. tuberculosis	12	24	7	14	
OPD	9	18	10	20	1.29
Asthma	18	36	11	22	0.52
BMI					
Under wt.	19	38	17	34	0.24
Ideal	14	28	16	32	0.884
Over wt.	17	34	17	34	
***M. tuberculosis* infection**					
Yes	23	46	8	16	10.52
No	27	54	42	84	0.001*

*BMI: Body Mass Index,*p = 0.005, industrial vs residential (n = 50 for each group)- see text for more details

Table 2a: Evaluation of different methods of assay for detection of *M. tuberculosis* in industrial area samples based on clinical findings

Assay parameter	Acid fast	Culture	PCR	ELISpot
Sensitivity (%)	80	93	94	93.8
Specificity (%)	100	100	100	100.0
PPV (%)	100	100	100	100.0
NPV (%)	25	30	75	60.0
Accuracy (%)	82	86	98	96.0

PPV: Positive Predictive Value, NPV: Negative predictive value

Table 2b: Evaluation of different methods of assay for detection of *M. tuberculosis* in residential area samples based on clinical findings

Assay parameter	Acid fast	Culture	PCR	ELISpot
Sensitivity (%)	69.4	86.1	97.2	88.9
Specificity (%)	100.0	100.0	100.0	100.0
PPV (%)	100.0	100.0	100.0	100.0
NPV (%)	28.0	73.7	93.3	77.8
Accuracy (%)	78.0	90.0	98.0	92.0

PPV: Positive Predictive Value, NPV: Negative predictive value

specificity, PPV, NPV and accuracy (%) were taken into account, where the results revealed that the molecular diagnostic methods were more sensitive than the traditional assays with higher NPV and accuracy (%). On the other hand, the specificity and the PPV (%) were the same for all methods. These results are in support to those reported by other researchers (Claudio and Claudio, 2003; Alexander *et al*., 2006), in which the diagnostic efficacy of the molecular methods is higher than the traditional assays. In this context, we evaluated the performance of the PCR using blood samples in comparison with ELISpot assay. Unsurprisingly, the efficacy of PCR assay was higher than the ELISpot assay, in a sense that the PCR technique is able to detect as little as 10 FG or the equivalent of 1 to 20 copies of *M. tuberculosis* complex genomic DNA. Accuracy of PCR in the present study reached up to 98% in both industrial and residential areas, while sensitivity of PCR detected in industrial area was 92 in comparison to 97.2% in residential area. The Accuracy of ELISpot assay dropped to 96% in industrial area in comparison to 92% in residential area, while sensitivity was 93.8% in industrial area and 88.9% in residential area. Indicating that ELISpot assay was as sensitive as PCR assay in detection of *M. tuberculosis* infection in industrial area, while in residential area PCR assay is more sensitive. These results were in agreement with previous data record by Jann-Yuan *et al*. (2007). Although previous studies demonstrated that the ELISpot assay for INF-γ is a powerful tool for detecting latent *M. tuberculosis* Infection (Alexander *et al*., 2006; Hadnagy *et al*., 1996) present results showed that in people who were previously vaccinated with BCG, the diagnostic value of this test in detecting active *M. tuberculosis* was less in percentage of sensitivity, NPV and accuracy, even in an area with expected high incidence of the disease. The performance of the PCR assay in this study was superior to that of the ELISpot assay because the probability of a wrong locus being mistakenly amplified twice is very low. Thus, false-positive results were decreased.

CONCLUSIONS

Air pollution, such as cement dust and smoke release from petroleum industries, has great impact on *M. tuberculosis* infection and spreading of the disease with the presence of other factors such as age, sex, family history and socioeconomic factors. Accurate diagnosis of *M. tuberculosis* is essential for disease control, however, the present study showed that detection of *M. tuberculosis* using PCR assay is more quick, less expensive, sensitive and can be used to detect *M. tuberculosis* DNA in sputum, blood and other body fluids, while ELISpot assay, although it is sensitive, is limited to detect presence of active *M. tuberculosis* infection in blood samples only. The present study recommend the use of PCR assay for *M. tuberculosis* detection and increasing the public awareness about air pollution and the positive importance of decreasing this type of pollution on human health. It is intend in the future to study the correlation between the degree of pollution and the corresponding infection.

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REFERENCES

Alexander, M.A., P.C. Hill, A. Fox, K.P.W.J. McAdam, D. Jackson-Sillah, M.D. Lugos, S.A. Donkor, R.A. Adegbola and R.H. Brookes, 2006. Reversion of the ELISPOT test after treatment in *Gambian tuberculosis* cases. BMC Infect. Dis., 6: 66.

Boyd, J.C. and J.J. Marray, 1975. Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. Ann. Intern. Med., 82: 489-492.

Boyd, R.F., 1995. Basic Medical Microbiology. 5th Edn. Lippincott Williams and Wilkins, ISBN-10:0316104450 pp: 330-339.

Chalupka, S., 2005. Environmental health: An opportunity for health promotion and disease prevention. AAOHN J., 53: 13-28.

Chang, S., L.S. Wheeler and K.P. Farrell, 2002. Public health impact of targeted tuberculosis screening in public schools. Am. J. Public Health, 92: 1942-1945.

Dye, C., C.J. Watt, D.M. Bleed, S.M. Hosseini and M.C. Raviglione, 2005. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence and deaths globally. JAMA., 293: 2767-2775.

El Demellawy, M.A., A. Abdel Wahab, E.M. Emad, K.M. Kandeel, A. Tabll and M.K. El Awady, 2006. Sensitivity of IS6110, mtp40 and 85B-RNA based amplification assays in the diagnosis and treatment follow up of pulmonary *Mycobacterium tuberculosis*. J. Biol. Sci., 6: 121-126.

Gupta, B., N. Mathur, P. Mahendra, A. Srivastava, V. Swaroop and M. Agnihotri, 1997. A study of household environmental risk factors pertaining to respiratory diseases. Energy Environ. Monitor., 13: 61-67.

Hadnagy, W., R. Stiller-Winkler and H. Idel, 1996. Immunological alterations in sera of persons living in areas with different air pollution. Toxicol. Lett., 88: 147-153.

Hill, P.C., R.H. Brookes, A. Fox, K. Fielding, D.J. Jeffries and D. Jackson-Sillah, 2004. 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., 38: 966-973.

Hill, P.C., A. Fox, D.J. Jeffries, D. Jackson-Sillah, M.D. Lugos and P.K. Owiafe, 2005. Quantitative T cell assay reflects infectious load of *Mycobacterium tuberculosis* in an endemic case contact model. Clin. Infect. Dis., 40: 273-278.

Jann-Yuan, W., C. Chien-Hong, L. Li-Na., H. Hsiao-Leng, J. I-Shiow, H. Po-Ren, Y. Pan-Chyr and L. Kwen-Tay, 2007. Diagnosis of tuberculosis by an enzyme-linked immunospot assay for interferon-γ. Emer. Infect. Dis., 13: 553-557.

Kaufmann, S.H., 2001. How can immunology contribute to the control of tuberculosis? Natl. Rev. Immunol., 1: 20-30.

Kennedy, N., S.H. Gillespie, A.O.S. Saruni, G. Kisyombe, R. McNerney, F.I. Ngowi and S. Wilson, 1994. Polymerase chain reaction for assessing treatment response in patients with pulmonary tuberculosis. J. Infect. Dis., 170: 713-716.

Maher, D. and M. Raviglione, 2005. Global epidemiology of tuberculosis. Clin. Chest. Med., 26: 167-182.

Metchock, B.G., F.S. Nolte and RJ. Jr. Wallace, 1999. Mycobacterium. In: Manual of Clinical Microbiology, Murray, P.R., E.J. Baron and M.A. Pfaller *et al*. (Eds.). 7th Edn. ASM Press, Washington, DC., pp: 399-437.

Mirza, S., B. Restrepo, J. McCormick and S. Fisher-Hoch, 2003. Diagnosis of tuberculous lymphadenitis using a polymerase chain reaction on peripheral blood mononuclear cells. Am. J. Trop. Med. Hyg., 69: 461-465.

Mishra, V.K., R.D. Retherford and K.R. Smith, 1999. Biomass cooking fuels and prevalence of tuberculosis in India. Int. J. Infect. Dis., 3: 119-129.

Murray, P.R., C. Elmore and D.J. Krogstad, 1980. The acid-fast stain: A specific and predictive test for mycobacterial disease. Ann. Int. Med., 92: 512-513.

Nicol, M.P., D. Pienaar, K. Wood, B. Eley, R.J. Wilkinson, H. Henderson, L. Smith and S. Samodien, 2005. Enzyme-linked immunospot assay responses to early secretory antigenic target 6, culture filtrate protein 10 and purified protein derivative among children with tuberculosis: Implications for diagnosis and monitoring of therapy. CID., 40: 1301-1308.

Perez-Padilla, R., C. Perez-Guzman, R. Baez-Saldana and A. Torres-Cruz, 2001. Cooking with biomass stoves and tuberculosis: A case-control study. Int. J. Tuberculosis Lung. Dis., 5: 1-7.

Pfyffer, G.E., B.A. Brown-Elliott and R.J.J. Wallace, 2003. *Mycobacterium*: General Characteristics, Isolation and Staining Procedures. In: Manual of Clinical Microbiology, Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (Eds.). 8th Edn. American Society for Microbiology Press, Washington, USA, pp: 532-559.

Piersimoni, C. and C. Scarparo, 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. J. Clin. Microbiol., 41: 5355-5365.

Raviglione, M.C., DE. Snider and A. Kochi, 1995. Global epidemiology of tuberculosis: Morbidity and mortality of a worldwide epidemic. JAMA, 18: 220-226.

Thomas, P. and J. Zelikoff, 1999. Air Pollutants: Moderators of Pulmonary Host Resistance Against Infection. In: Air Pollution and Health, Holgate, S.T., J.M. Samet, H.S. Koren and R.L. Maynard (Eds.). CA, Academic Press, San Diego .

Wang, J.Y., L.N. Lee, C.S. Chou, C.Y. Huang, S.K. Wang and H.C. Lai, 2004. Performance assessment of a nested-PCR assay (the RAPID BAPMTB) and the BD ProbeTec ET system for detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol., 42: 4599-4603.