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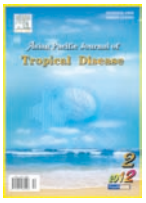

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SEVA TB ELISA – Multi antigen and antibody assays for serodiagnosis of suspected cases of pulmonary and extra pulmonary tuberculosis in tertiary care hospital –A retrospective study

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ABSTRACT

Objective: To assess the usefulness of in-house developed multiantigen and antibody assays, in diagnosis of both pulmonary and extra-pulmonary tuberculosis. **Method:** Clinically suspected cases of 31 pulmonary and 171 extra-pulmonary tuberculosis (TB) were screened by ELISA using cocktail (ES-31 + EST-6) antigen and their specific antibodies (anti ES-31 + anti EST-6 IgG) for detection of antibody and and antigen (circulating antigen and immune complexed antigen) respectively and correlated with antituberculosis therapy in retrospective study. **Results:** Out of 31 cases of pulmonary TB screened, 15 patients showed ELISA positivity out of which five cases were given antituberculosis therapy. Out of 171 cases of EPTB screened, 76 cases showed ELISA positivity out of which 18 were given antituberculosis therapy. Further 4 EPTB cases which showed AFB negativity were given ATT. The data was further analyzed based on PTB & EPTB, adults and children, OPD and IPD patients to understand false positivity in clinically suspected PTB and EPTB cases. There was significant correlation (108/202 cases) with ELISA negativity and no ATT advised in clinically suspected PTB and EPTB patients. **Conclusions:** In house developed multi antigen and antibody assays have been observed to be quite useful as adjunct test in serodiagnosis of suspected cases of tuberculosis in particular extrapulmonary tuberculosis.

1. Introduction

Overall one-third of the world's population is currently infected with tuberculosis bacillus. World Health Organization estimates that India account for one fifth of total global TB burden [1]. Up till now there was known burden of pulmonary tuberculosis. Recently there has been emergence of threat of extra pulmonary tuberculosis. Data from USA clearly shows that rate of pulmonary tuberculosis are declined with relative increase in extra pulmonary TB cases. This global increase in tuberculosis is believed to be due to HIV related immuno-incompetence[2]. Early diagnosis of tuberculosis and initiating optimal treatment is a key to control disease progression and to prevent its spread. To achieve this goal, many programs are planned for its control and treatment. Recently World Health Organization has advised governments to ban commercial TB diagnostic tests for poor sensitivity and specificity in detecting pulmonary and extrapulmonary tuberculosis. Demonstration of acid fast bacilli and culture has been a gold standard in diagnosing tuberculosis in spite of low sensitivity or delay in detection. Moreover visualization of AFB in direct smears by Zeil-Nelson staining requires bacillary densities of >10,000 bacilli/ml and therefore limits its sensitivity [3]. Detection of AFB in culture also has low sensitivity of 30-40%. With improved version of BACTEC rapid culture, sensitivity of 90% and specificity of 95% has been achieved but it also requires expert technicians and is expensive[4]. Another popular diagnosing aid, 'X-ray Chest' can not

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differentiate pulmonary lesion from other pathological conditions and also question arises in TB with non-classic presentation like in extrapulmonary tuberculosis [5]. Among recent adjunct methods in TB diagnosis tests like interferon gamma release assay limit themselves with low sensitivity in immune compromised individuals [6]. Apart from limitation in diagnosing aids, question also arises in sample collection. As TB has differential ways of presentations, particularly when extra-pulmonary tuberculosis is concerned. Problem in identifying exact site for sample collection, finding of AFB bacilli in them or culture them, are the real challenges faced by clinicians and laboratory personnel. Due to lack of exact guidelines for sample collection, it involuntarily delays in diagnosis and hence in treatment. Further difficulty of getting sputum sample in children makes the sputum smear not practicable. Due to all these reasons, it necessitate to develop a sensitive, reliable, simple and cost effective test for diagnosis of tuberculosis both pulmonary and extra pulmonary in active form provided that test should not depend on its immunological status of person in particular HIV cases. Serological tests have a potential of providing inexpensive and robust tool for tuberculosis diagnosis [7]. Over decade's lots of emphasis has been put for serodiagnosis and ELISA system and was found to be appropriate approach. Serological tests are simple to use, inexpensive and easy to interpret as they do not depend on the site of infection and are not only suitable for pulmonary cases but also extra-pulmonary cases and in uncooprative, paediatric cases. In earlier studies from our laboratory we have reported, the diagnostically important antigens that are ES-31, ES-41, and ES-43 in antibody detection by penicillinase ELISA. A cocktail of ES-31, ES-41 and ES-43 antigens has shown improved sensitivity when compared with single ES-31 antigen antibody detection in PTB [8]. All these penicillinase based ELISAs are sensitive but they are semi-quantitative and subjective. Another improved microtitre plate Peroxidase sandwich ELISA for detection of ES-31 antigen was reported form this laboratory for diagnosis of tuberculosis patients [9]. We had conducted a retrospective study of 6 months to analyze usefulness of peroxidase ELISA using cocktail antigen correlated well with AFB positivity and ATT treatment in tertiary care hospital. However significant number of cases did show ELISA positivity but not ATT treated [10]. In this retrospective 9 months (October 2010 to June 2011) study we further studied the usefulness of in-house developed SEVA TB peroxidase enzyme immuneassay using multi-antigen (ES-31 & EST-6) in antibody detection and affinity purified antibody (antiES-31 & anti EST-6 containing ES-41 and ES-38) assays in antigen detection for diagnosis of pulmonary and extra-pulmonary tuberculosis in correlation with AFB smear test and ATT treatment by further analysis of patients classified as PTB and EPTB, adults and children examined in out patient or inpatient department.

2. Materials and Methods

This retrospective study was carried out at Mahatma Gandhi Institute of Medical Sciences, with a tertiary Rural Hospital located at Sevagram, Maharashtra, India. Blood samples were received for in house developed ELISA test from suspected patients of tuberculosis on clinical grounds and other laboratory investigations. Total samples collected were 202. The study group includes both category of tuberculosis i.e. pulmonary (31) as well as extra-pulmonary (171) cases. These samples in each category were further divided into adults' cases and children (Table-1). Under a category of extra-pulmonary tuberculosis we have cases from TB bone and joint (107), abdominal TB (31), lymphnode TB (12), genitourinary TB (13), ocular TB (5), and TB meningitis (3) (Table-1). Serum from these clinically suspected cases were screened by ELISA using cocktail (ES-31 + EST-6) antigen and specific antibodies (anti ES-31 + anti EST-6 IgG) for detection of antibody and circulating free and immune complexed antigens respectively and correlated with anti tuberculosis therapy.

2.1 Isolation of mycobacterial ES-31 and EST-6 antigens and their antibodies:

Mycobacterial detergent-soluble sonicate antigen (DSS Ag) was prepared from phenol(5%) inactivated M. tuberculosis H37Ra bacilli[11]. Briefly, ten loopful phenol (5%) inactivated bacilli were suspended in 4 ml of 0.05 M phosphate-buffered saline (PBS) (pH 7.2) and then sonicated for 30 min with 30sec bursts and one min interval. The sonicate was incubated in boiling water bath with 2 ml of sodium dodecyl sulfate (SDS) extraction buffer (5% SDS, 5% 2-mercaptoethanol, and 8 M urea in 0.01 M PBS, pH 7.2) for 5 min, followed by incubation at 480C for 24 h. The supernatant was separated, dialyzed against 0.01M PBS, pH 7.2, for 48h, concentrated and labeled as DSS Antigen. Antibodies (Anti-DSS-IgG) against DSS antigen were raised in goat by injecting 500 μg protein/ml DSS antigen and 1ml of Freund's incomplete adjuvant intramuscularly on days 0, 20, 33, and 45. Immune sera were collected on days 32, 44, 57 and 60. Immunoglobulins from sera were precipitated with 33% ammonium sulfate to isolate Anti-DSS IgG by diethylaminoethyl-cellulose ion exchange column chromatography.

ES-31 antigen was isolated from M. tuberculosis H37Ra culture filtrate (ES) antigen by affinity chromatography using anti-ES-31 antibody-coupled Sepharose-4B column [12]. EST-6 antigen containing mixture of ES-41 & ES-38 antigens was obtained by 6% trichloroacetic acid (TCA) precipitation of M. tuberculosis ES antigen, followed by SDS-PAGE fractionation. EST-6 antigen was eluted from sixth gel fraction. Anti-ES-31 antibody was isolated from anti-DSS IgG by affinity chromatography [11]. Briefly, Anti-DSS IgG was passed through the anti-ES-31 antibody-

coupled Sepharose-4B column and anti-ES-31 antibody was eluted by glycine-HCl buffer (0.01 mol/L, pH 2.5) and collected in Tris-HCl buffer (0.01M, pH 8.6). Similarly, anti-EST-6 antibodies were isolated from anti-DSS IgG by affinity chromatography using EST-6 antigen-coupled Sepharose-4B beads. Cocktail antigen (ES-31 and EST-6) and antibody (anti-ES-31, and anti-EST-6) were prepared by mixing the antigens and antibodies in equal proportion.

2.2 Enzyme Linked Immunosorbent Assays(SEVA TB ELISAs) for cocktail antibody, circulating free and IC-antigen:

Indirect peroxidase ELISA for detection of antibody was performed using cocktail of ES-31 and EST-6 antigens[10]. Briefly, the wells of ELISA plate (NUNC) were sensitized with cocktail antigen (2 μg/well) in 0.06M carbonate buffer pH9.6 overnight at 40C, followed by blocking with 2% BSA for 1hr at 370C. Plates were washed twice with PBS/T (PBS containing 0.05% Tween20) followed by addition of sera (1:50 dilution) in PBS/T for 1h at 370C, then washed 3 times. After that the wells were incubated in 1:15000 diluted rabbit-anti-human-IgG peroxidase conjugate for 1h at 370C. The wells were again washed thrice with PBS/T. The color was developed using TMB substrate (20X concentration) and 50 μl 2N H2SO4 was used to stop the reaction. Then mean optical density at 450nm was read with ELISA reader.

Sandwich ELISA for the detection of circulating free cocktail antigen (ES-31 and EST-6) was performed using anti-cocktail antibody (anti-ES-31 and anti-EST-6) [9]. Briefly, the plates were sensitised with anti-cocktail antibody 100 μg/well, followed by blocking, incubation with sera (1:50 dilution) in PBS/T for 1h at 370C and finally incubating the wells with goat anti-cocktail(anti-ES31 and anti-EST-6)- antibody-IgG-HRPO conjugate. TMB was used as a substrate and 2N H2SO4 to stop the reaction.

3. Results

During study 202 (31 pulmonary and 171 extrapulmonary) TB suspected sera samples were screened by peroxidase immunoassay using cocktails of antigens and antibodies (Table 1). Details of ELISA results and ATT treatment in cases of pulmonary and extrapulmonary TB from IPD or OPD and adults or children were given in Table 2. Table 3 shows that the consideration of presence of either of Ab, Ag or IC-Ag shows better correlation with ATT compared to antibody or antigen positivity alone.

Out of total 31 suspected pulmonary cases, only 15 cases of pulmonary tuberculosis were advised for acid fast bacilli culture. Only one case of PTB was reported to be AFB positive which was also ELISA positive and was started on

Table 1

Multi-antigen and antibody assays for serodiagnosis of suspected cases of pulmonary and extrapulmonary tuberculosis in a tertiary care hospital during Oct 2010 to June 2011.

Group	TotalNo. Screened*	Adult			Children		
		Total adults	ELISA positive**	ELISA Negative	Total children	ELISA positive**	ELISA Negative
Pulmonary TB	31	23	11	12	8	3	5
Extrapulmonary TB	171	148	67	81	23	9	14
Bone and Joint TB	107	99	47	52	8	3	5
Abdominal TB	31	26	11	15	5	2	3
Lymphnode TB	12	6	3	3	6	2	4
Genitourinary TB	13	12	6	6	1	1	–
Occular TB	5	5	–	5	0	–	–
TB Meningitis	3	0	–	–	3	1	2

* Out of 202 cases, 24 cases (PTB-15 and EPTB-9) were screened for AFB positivity. Only one PTB patient was AFB positive, ELISA positive and ATT given.

** ELISA Test was done using cocktail antigen (ES-31 Ag + EST-6 Ag) and cocktail antibody (Anti-ES-31 + Anti-EST-6) for antibody and antigen (free and immune-complexed antigen) detection respectively. ELISA test positive either for antibody or free antigen or immune-complexed antigen is considered as ELISA positive.

Table 2

Analysis of ELISA positive and negative cases and correlation with antituberculosis treatment.

Group	Total *(ATT treated)	Total ELISA positive	ELISA positive cases				Total ELISA negative	ELISA negative cases			
			Adult TB		Childhood TB			Adult TB		Childhood TB	
			OPD	IPD	OPD	IPD		OPD	IPD	OPD	IPD
Pulmonary TB	31(5)	14(5)	6(0)	6(3)	0(0)	2(2)	17(0)	10(0)	1(0)	3(0)	2(0)
Extrapulmonary TB	171(22)	76(18)	25(1)	44(13)	1(0)	5(4)	95(4)	22(2)	67(1)	4(0)	1(1)

* Figures in parenthesis indicate the cases given anti tubercular therapy.

OPD: Out Patient Department, IPD: In Patient Department.

Table 3

Status positivity of Ab, Ag and IC-Ag in ATT given cases.

Sr. No	CR No.	Clinical Diagnosis And other investigation	Other investigation	Ab	Ag	IC-Ag	Ab/Ag/IC-Ag
PTB cases							
1	201012046751	Left sided pleural effusion? TB with sickle cell trait.	AFB -ve	+	-	+	
2	201010098622	Left sided pleural effusion	X-ray chest- massive effusion	+	-	+	+
3	201010086829	Left sided pleural effusion (DOTS-I)	ADA(73) +ve, X-chest reveals pleural effusion	+	-	-	+
4	201010095941	Right sided pleural effusion	B-20 -ve, X-chest reveals pleural effusion	+	+	+	+
5	201101012941	Polythralgia with pulmonary tuberculosis	RA +ve	-	+	-	+
Total				4	3	2	5
EPTB cases							
1	200908010808	Abdominal TB (DOTS-I), low back ache		-	+	-	+
2	201011009860	Tuberculosis spine AKT4 for 3 months		-	+	-	+
3	201011011891	Ischemic Disease prolapsed invertebral disc? tuubercular		-	-	-	-
4	201011019777	TB right knee with fixed flexion deformity AKT for 1 month	AFB -ve	-	+	-	+
5	201011025361	Tuberculosis C4-C5 AKT for 1 month		+	+	-	+
6	201011075332	Abdominal Koch (DOTS-I)		+	+	+	+
7	201012049297	RIIH with left orchitis tuberculosis		+	-	-	+
8	200712074404	Cyopathology smear shows marked lymphocytosis consistant with tubercular effusion		+	-	-	+
9	201012076952	Pots spine L5 level with milliary TB	AKT for 4 months AFB-ve	+	+	-	+
10	201012112940	Prolapsed invertebral disc, bilateral laminar pleural effusion		-	-	-	-
11	201102066087	Low backache with neurodefict with B/L LL pain with tubercularpsoas abscess		-	+	+	+
12	201101095359	Acute meningoencephalitis with infarct and communicating sequel to meningitis cause ? tuberculosis etiology. Cytology fluid smear shows marked lymphocytosis	Mantaux test -ve	-	-	-	-
13	201101106105	Tubercular right hip arthritis, right hip joint effusion		-	-	-	-
14	201102055903	Tuberculosis C3-C4 vertebrate cord compression and quadraparesis and hypertension old		+	-	-	+
15	201102090431	Left knee osteomylitis ? tubercular		+	-	+	+
16	201103023800	tubercular sinuvitis lt elbow		+	-	-	+
17	201103046691	Abdominal tuberculosis		+	-	-	+
18	201103123511	tubercular right hip joint		+	+	-	+
19	201105039410			+	-	+	+
20	201106081577	x-ray cervical spine ap/lat potts spine C2C3 potts spine cervical with tubercular abscess		-	+	-	+
21	201106021469	D12 fracture vertebra with paraparesis cause tuberculosis		+	+	+	+
22	201104082460	sinuvitis right elbow? tubercular		+	-	-	+
Total				13	11	5	18

ATT. Thus ELISA showed correlation with AFB positivity and ATT. Out of 31 PTB cases 14 were ELISA positive out of which 5 (3 adult and 2 children) were given ATT including one AFB positive case. Thus ELISA supported the clinician in 4 cases to decide on clinical grounds to start ATT in absence of AFB smear test. 9 pulmonary cases were ELISA positive and not advised for ATT were to be followed up. All the five PTB cases treated were from in patient department under supervision of clinician. Increased false positivity was observed in OPD cases. 17 cases were ELISA negative and not advised ATT. ELISA showed 100% co-relation with ELISA negativity and no ATT for pulmonary tuberculosis.

Out of 171 EPTB cases 76 were ELISA positive. 9 EPTB cases were screened for AFB and none was AFB positive. Out of 76 ELISA positive cases 18 (14 adults and 4 children) were recommended for ATT. Thus here also ELISA strongly supported clinician in confirming diagnosis of extrapulmonary tuberculosis and to start ATT showing 100% correlation with ELISA positivity and ATT treatment. OPD cases showed more false positivity compared IPD cases. Details are given in Table 3. Out of 95 suspected EPTB cases which were ELISA negative, 58 cases showing ELISA positivity were not advised ATT. Only four cases were advised ATT showing good correlation of ELISA negativity with absence of disease. A preliminary study on analysis of few ELISA positive sera of suspected EPTB patients which were not advised ATT did show the presence of ES-31, ES-43 and ES-41 antigens showing TB infection[14] justifying

the need for follow up of these cases for developing clinical disease in due course of time. Such ELISA positive cases needed to be justified whether to start ATT depending on the severity of symptomatology like chronic weight loss, chronic cough not getting relieved with short term treatment of higher antibiotics, fever and other blood investigations etc.

4. Discussion

Though traditional methods of TB diagnostics like AFB microscopy and culture have key role in confirming tuberculosis but may not be useful for lack of sensitivity and considerable time required in particular in suspected TB cases referred to tertiary hospital. Similarly many obstacles are present in diagnosis beginning from its presentation, sample collection, clinical co-relation and finally treatment. Not all TB cases show classical presentation in particular extra-pulmonary tuberculosis. Because of its number of ways of presenting symptoms leading to difficulty in sample collection, again wrong specimen selection, insufficient quantity of specimen, contaminated specimen etc. making it difficult task for laboratory diagnosis. It gives stressful dilemma to clinician whether to start ATT treatment or not. Problem arises in childhood tuberculosis as there is difficulty in sample collection in both cases that is pulmonary as well as extra-pulmonary. To be sure in confirming diagnosis it needs such test which will overcome all these problems. Immunologocal tests are the best answer for diagnosis and are also cost-effective. Usefulness of SEVA TB ELISA has been reported from our laboratory in confirmation of TB in clinically diagnosed and ATT advised cases. Over a decade, our laboratory has been using excretory-secretory antigens and specific antibodies in TB diagnosis. An assay detecting free circulating cocktail antigen was found to be 91% sensitive and 97% specific for sputum positive cases and also in detection of EPTB cases [13]. As penicillinase assay is subjective and semiquatitative, as a result microtitre plate peroxidase sandwich ELISA was explored by using affinity purified anti ES-31 antibody for detection of circulating antigen in TB sera [9] with sensitivity of 80% and 90% of specificity. In the absence of affordable and reliable commercial test, EPTB cases and children suspected of TB are mostly treated based on clinical diagnostic criteria and chemotherapeutic trial. In-house developed multi-antigen and antibody ELISA (SEVA TB ELISA) has been observed to be useful as an adjunct test, in confirming TB in clinically difficult diagnosis cases and ruling out TB in suspected cases of pulmonary and extra pulmonary tuberculosis avoiding unnecessary and toxic anti tuberculosis therapy [10].

In present study ELISA has shown co-relation with AFB positivity and ATT treatment. ELISA has supported in starting of ATT in TB suspected but AFB negative patients in pulmonary tuberculosis. 5 cases of pulmonary tuberculosis were advised ATT, out of which only one was AFB positive where as all five PTB cases were ELISA positive. Reason for AFB negativity may be of sensitivity limitations of sputum microscopy and mycobacterial culture requiring >10,000 bacilli/ml of sputum [3]. All these 5 cases were from indoor department. Remaining 9 cases which were ELISA positive and not advised ATT were OPD patients. Indoor patients are easy to counsel and easy to reach to proper diagnosis and follow up. All ELISA positive 9 OPD patients may not visit OPD again and needs to be followed up. ELISA positivity for these subjects may be because they have only TB infection and not yet developed into disease [15].

ELISA was found to be more helpful in extra-pulmonary tuberculosis cases. In the absence of affordable and reliable commercial test EPTB cases and children suspected of TB are mostly treated based on clinical diagnostic criteria and chemotherapeutic trial. In-house developed multi-antigen and antibody ELISA (SEVA TB ELISA) has been observed to be useful as an adjunct test, in confirming TB in clinically difficult diagnosis cases and to avoid unnecessary toxic anti tuberculosis therapy [10]. From the data it is clear that ELISA load was high for EPTB (51 PTB Vs 171 EPTB) in these 9 months study. There is 100 % correlation with ELISA positivity and ATT treatment. All 18 cases which were ELISA positive had received ATT (Table 1). 91 out of 95 suspected cases showed ELISA negativity and were not advised ATT. 18 ELISA positive patients to whom ATT was started were from indoor and one was from OPD. Remaining 58 patients which were ELISA positive and not ATT received were needed to be followed up. It is essential to mention here about those cases which were clinically suspected of TB though showed ELISA positivity and were not advised ATT. This is possibly because that these subjects have only TB infection and not yet developed into TB disease. This is the period called latent TB. Such populace needs to be followed up. Almost 5% - 10% of these subjects may develop into TB disease in future [15]. Hence such patients may be advised to visit OPD again unless clinical symptoms of suspicion like swelling or pain in bone or any soft tissue with considerable weight loss and fever disappear or persistence of symptoms even after short term course of broad spectrum antibiotics. In preliminary study ELISA patients showing ELISA positivity but not advised ATT were screened for presence of antigen by immunoblotting. Such ELISA positive cases showed the presence of antigens (ES-31, ES-43 and ES-41) while they were absent in ELISA negative sera [14]. Thus SEVA TB ELISA has been helpful in finding a subject in latent phase and is needed to pay attention in coming days.

There were four cases out of 95 clinically suspected cases of EPTB (2 in OPD and 2 IPD) which were ELISA negative and were treated with ATT. One pediatric case was of suspected tubercular meningitis and was started with ATT in correlation with symptoms and other laboratory investigations like fluid cytology which showed marked lymphocytosis (Table-3). Other three cases were treated on

the basis of clinical symptoms and MRI findings suggestive of tuberculosis. ATT was started in those patients with positive MRI findings suggestive of tubercular effusion. These cases needed to be followed up.

As MGIMS is located in rural area, people referred to this tertiary level of health delivery system insist for early and proper diagnosis with accurate treatment. They can not afford to lose time which will aggravate the clinical condition. As there is no reliable commercial test is available, the clinician requests for SEVA TB ELISA, an inhouse developed ELISA for confirming clinical suspicion and starting ATT. Thus SEVA TB ELISA has been helpful as supportive test to diagnose AFB negative PTB and EPTB cases. Such serodiagnosis will save not only time but also aggravation of disease manifestations. It also identifies risk group of clinically suspected population which have risk of developing tuberculosis in coming months or years. Thus, inhouse developed, less expensive, user friendly peroxidase ELISA has been used as an adjunct test in confirming tuberculosis along with smear microscopy or culture techniques for routine screening of suspected cases of PTB and EPTB for better detection and management.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] World Health Organization. Global tuberculosis control- a short update to the 2009 report.p4-5. [Online available] from http://www.who.int/tb/publications/global_report/2009/update/tbu_9.pdf.

[2] Sarman Singh & V M Katoch. Commercial serological tests for the diagnosis of active tuberculosis in India: Time for introspection. *Indian J Med Res* 2011; **134**: 9583-587.

[3] Harinath BC, Immunodiagnostics for Tuberculosis-Problems and Progress. *Ind. Jr. Tuber.* 2010; **57**:p123-127.

[4] Rendong Fang Xia Li, Lin Hu, Qimin You, Jing Li. Cross-Priming Amplification For Rapid Detection of Mycobacterium Tuberculosis in Sputum Specimens. *J Clin Microbiols* 2009, p845-847.

[5] Anastasios K. Testing for tuberculosis Aust. Prescr 2010;33:p12-18.

[6] Markova R, Todorova Y, Drenska R, Elenkov I, Yankova M, Stefanova D. Usefuless of Interferon-Gamma Release Assay in the Diagnosis of Tuberculosis Infection in HIV-infected Patients in Bulgaria. Biotechnol & Biotechnol Eq.23/2009/1;p1103-1108.

[7] Sagrika Haldar, Mridula Bose, Parul Chakrabarti, Hatim F. Daginawala, B.C.Harinath, Rajpal S Kashyap, Savita Kulkarni, Anindita Majumdar, H. Krishna Prasad, Camilla Rodrigues, Ranjana Shrivastava, Girdhar M. Taori, Mandira Verma- Basil, Jaya S. Tyagi. Improved laboratory diagnosis of tuberculosis-The Indian Experience.Tuberculosis.Sept. 2011;91(5):p414-426. (PMID:21764383)

[8] Gupta S, Shende N, Kumar S, Harinath BC. Detection of antibodies to a cocktail of mycobacterial excretory secretory antigens in tuberculosis by ELISA and immunoblotting. *Curr Sci* 2005; **88**: p1825-7.

[9] Majumdar A, Upadhye V, Harinath BC. Peroxidase enzyme immunoassay for circulating SEVA TB ES-31 antigen in pulmonary tuberculosis sera. *Biomed Res* 2008; **19**(3): p201-6.

[10]Anindita M, Pranita D. Kamble, CM Badole, BC Harinath. Prospective study of SEVA TB peroxidase assay for cocktail antigen and antibody in the diagnosis of Tuberculosis in suspected patients attending a tertiary care hospital located in rural area. *Asian Pac J Trop Med* 2010; p356-359.

[11]Harinath BC, Kumar S, Roy SS, Hirudkar S, Upadhye V, Shende N. A cocktail of affinity purified antibodies reactive with diagnostically useful mycobacterial antigens ES-31, ES-43 and EST-6 for detecting the presence of Mycobacterium tuberclosis. *Diag Microbio Inf Dis* 2006; **55**: p65-8.

[12]Nair ER, Banerjee S, Kumar S, Reddy MVR and Harinath BC. Purification and characterization of a 31kDa mycobacterial excretory - secretory antigenic protein with a diagnostic potential in pulmonary tuberculosis. *Ind J Chest Dis Allied Sci* 2001; **43**: p81-90

[13]Upadhye V, Shende N, Kumar S, Harinath BC. Detection of antibody and antigen in extrapulmonary tuberculosis patient's sera using a cocktail of mycobacterial excretory secretory antigens and their antibodies. *Biomed Res* 2007; **18**:p161-6.

[14]Majumdar A. Studies on mycobacterial excretory- secretory protein antigens of diagnostic interest in HIV-TB coinfection-Ph.D. Thesis.2011.p131-132.

[15]Centers for disease control and prevention. TB Elimination. The Difference Between Latent TB Infection and TB Disease. 2011. [Online available from : http:/www.cdc.gov/tb/publications/factsheets/general/LTBI and Active TB pdf .]