Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

CLINICAL UTILITY OF INTERFERON- γ RELEASES ASSAYS (IGRAS) IN POTT'S DISEASE

Manoj Kumar[1], Raj Kumar[2], AK Srivastava[2], VL Nag[3], AK Maurya[3], N Krishnani[4], TN Dhole[3] and Sunil G Babu[1*]
[1]Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, India
[2]Department of Neurosurgery, [3]Department of Microbiology, [4]Department of Pathology,
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Received for publication: December 19, 2012; **Revised**: January 12, 2013; **Accepted**: February 21, 2013

Abstract: Interferon-γ release assays (IGRAs) promised a sensitive and specific diagnosis of latent and active infections of Mycobacterium tuberculosis. Therefore, the intent of this study was to appraise the clinical usefulness of Quantiferon-Gold in Tube (QFT-GIT) for diagnosis of Pott's disease. We prospectively observed 55 clinical radiological suspected cases of Pott's disease. They were undergone to the detection of tubercular infection by QFT-GIT, microscopy, culture, polymerase chain reaction (PCR) and histopathological methods. The sensitivity and specificity of QFT-GIT was calculated against the combined results of performed tests. Patients classified into 2 groups as per anti tubercular treatment (ATT), 24 (43.6%) were new cases (group A) and 31 (56.4%) were relapse / on treatment cases (group B). The positivities among group A and B were; 21 (87.5%) and 19 (61.3%) on QFT-GIT, 10 (42%) and 10 (32.2%) on microscopy, 14 (58.3%) and 13 (54%) on culture, 19 (79.2%) and 18 (58%) on PCR, 21 (87.5%) and 18 (58%) on histopathology. The significant positivities differences (p value <0.05) among group A and B were found on QFT-G IT and histopathology. Moreover, we found 45 cases as a definite TB and 10 cases were negative by all tests. Out of these 45 definite TB, 38 cases and out of 10 negative cases two cases detected positive by QFT-GIT. QFT-GIT revealed higher sensitivity in new cases when compared to retreated cases. Its results are also coinciding the combined results of laboratory proven methods and this test is useful in diagnosing of Pott's disease with 84% sensitivity.

Keywords: IGRAs, Pott's disease, *M. tuberculosis* and ATT

INTRODUCTION

Pott's disease is a form of extra pulmonary tuberculosis (EPTB) and its frequency is 50% of the cases of skeletal tuberculosis, 15% of the cases of EPTB and 2% of all cases of tuberculosis (TB) (1). The neurological complications are the most crippling complications of this disease. Their incidence ranging from 10% to 43% in various previous reports. The case fatality remains relatively high, even the availability of an effective anti-tubercular treatment (ATT) regimens (2), (3), (4). For the reason that, most of the cases not diagnosed at an early stage of this disease particularly in our country. Hence, an early and accurate diagnosis required for prescription of adequate treatment, it is foremost factor for a constructive prognosis and the prevention of long-term neurological sequel of Pott's subjects.

The diagnostic battery of Pott's disease includes microbiological and typical pathological examinations. They are either time consuming or lack of desired sensitivity (5), (6). Further, nucleic acid based (molecular) diagnosis is quick and has higher sensitivity but they have reached away from rural areas and requires expertise manpower (7), (8). All above available diagnostic modalities requires specimens from invasive procedure for detection of definite tubercular infection of the spine. (9) However, immunological methods using a specific humoral or cellular responses of the host to infer the presence of infection or disease and does not require the specimen from the site of infection and invasive procedure. (9)

Despite, the tuberculin skin test (TST) suffers with low specificity in BCG vaccinated individuals, a high interobserver variance and requires skill to be read and interpreted. (10) Thus, a new diagnostic method is required to overcome the ambiguity in the detection of tubercular infection.

QuantiFERON-TB-Gold In-Tube (QFT-GIT) (Cellestis Ltd, Victoria, Australia) is a commercial test which depends on the interferon gamma release assays (IGRAs). It is based on the T-cells sensitized with tuberculous antigens. They produce IFN-γ when they are re-exposed *ex vivo* to mycobacterial antigens; a high amount of IFN-γ production is then presumed to correlate with TB infection (11), (12). The current IGRA use the early secretory antigenic target 6 (ESAT-6), and the culture filtrate protein 10 (CFP-10); both proteins are coded by genes located in the region of difference 1 (RD1) of the *Mycobacterium tuberculosis* (*M. tuberculosis*) genome and are not shared with *M. bovis* BCG or most nontuberculous mycobacterias (NTM), with the exception of *M. marinum, M. sulzgai and M.*

***Corresponding Author:**
Dr. Sunil G Babu,
Department of Biotechnology,
Babasaheb Bhimrao Ambedkar University,
Lucknow,India
Email add: sunil_gos@yahoo.com

kansasii (12). QFT-GIT having a additional third specific antigen TB7.7, encoded by a phage-inserted region (phiRv2) which is highly specific for *M. tuberculosis* (13). Herein, the intent of the present study was to assess the clinical usefulness of QFT-GIT tests on new and retreated cases and their significance against a diagnostic battery of Pott's disease for establishing an initial test for this disease at a tertiary care hospital, north India.

MATERIALS AND METHODS

A total of 55 suspected cases (clinical radiological examinationed) were enrolled in this study from June 2008 to July 2011 in the Department of Neurosurgery, at Sanjay Gandhi Postgraduate Institute of Medical Science Lucknow, India. Information about prior intake ATT/ tubercular history was based on self-declaration of patients. Two ml blood specimen was collected from each individual for QFT-GIT and tissue/ pus specimens collected from either during surgical or by CT guided fine needle aspirates (FNA). The definite tubercular infection recognition based on the prospective examinations of clinical specimens (pus/tissues) on ZN microscopy, BACTEC culture, PCR and histopathology were carried out.

QuantiFERON-TB Gold in *tube* (QFT-GIT):

QFT-GIT measured to IFN-γ concentrations induced by *M. tuberculosis* specific antigens (TB-Antigen) for detecting TB infection. The ELISA based IGRAs (QFT-GIT), Cellestis, Victoria, Australia' directly one ml whole blood was collected in the TB antigen tube and another one ml in the nil tube for negative control. The blood was mixed in the tubes and placed in the incubator at 37°C for 18 hours. Approximately 200 µl of plasma were harvested from each tube after centrifugation. IFN-γ concentrations in the plasma were measured by ELISA, using Microtiter plate reader (TECAN, Sunrise A-5082 grading Salzburg, Austria). The obtained results were analyzed at the cutoff value (≥0. 35, IU/ml) as recommended by the manufacturer using provided software (QFT-GIT Analysis Software, ver. 2.50, Cellestis Limited, Australia).

Ziehl Neelsen (ZN) Microscopy and BACTEC Culture:

Microscopic smears were made by ZN stain according to standard laboratory procedures. (14) Culture was done on radiometric BACTEC 12B vials. The vials were incubated and interpreted as per Becton Dickinson (BD, Sparks, MD, USA) manual instructions. (15) The p-Nitro-α-acetylamino- β-hydroxy propiophenone (NAP) test was performed to the identification and differentiation of Mycobacterium tuberculosis complex (MTBC) from non-tubercular *Mycobacterium* (NTM) in all grown isolates. (15)

DNA extraction from pus:

Genomic DNA was extracted from pus specimens according to Van Soolingen et al (16) described previously. The tissues were processed by Hipura genomic DNA extraction kit according to the manufacturer protocol, Himedia laboratories Private Limited, India.

PCR- Primers and PCR amplification:

The amplification reaction was performed in a final volume of 20 µl for each specimen. The reaction mixture contained 10 µl Pyrostart Fast PCR Master Mix 2X (dNTP, Taq polymerase with $MgCl_2$,), 1µl (10 pmole) of each primer, 3 µl water (nuclease free) and 5 µl of extracted genomic template DNA according to Fermentas India. The oligonucleotide primers (17) used were forward and reverse are: 5'-CCT GCG AGC GTA GGC GTC GG3' and 5' CTC GTC CAG CGC CGC TTC GG 3' respectively (SBS Gentech Co. Ltd). These primers were amplified a target fragment (123 bp) of the repeated insertion sequence *IS6110* of MTBC.

The PCR amplification was done in a thermal cycler (MJ Research, PTC-100, GMI, Inc, USA). In brief, the initial denaturation was done at 94°C for 5 min. Further, amplifications were subjected to 35 cycles of PCR. Each cycle was preceded by denaturation at 94°C/ 2 min, annealing at 68°C /2 min and extension at 72°C /1min followed by a final extension at 72°C for 7 min was carried out.

An aliquot (10 µl) from the PCR amplified product was analyzed in 2% agarose gel through electrophoresis in Tris- acetate EDTA (TAE) buffer for 40 minutes at 95Volt. The gel was stained with ethidium bromide and visualized on the UV - transilluminator. The presence of a 123 bp fragment indicated a positive test with respect of positive control. Each PCR series had one positive control (50-100 pg H37 Rv DNA) and one negative controls (RNAs and DNAs free water) interpreted with the specimens to monitor cross- contamination.

Histopathology:

The tissues stained with hematoxylin Eosin (HE) and ZN stain were analyzed under microscope for epithelioid cell granulomas with or without the presence of Langerhans giant cell and acid fast bacilli (AFB). (18)

Subjects group classification:

A) QFT-GIT was done in all subjects at the time of admission

i. **Group A** - subjects who have no any past history of TB or ATT.
ii. **Group B** - Relapse, retreated, refractory or continued intake ATT for more than one month.

B) Disease status according laboratory findings

i. **Definite TB –** At least positive by one performed method, either on ZN microscopy /BACTEC culture/PCR *IS6110* or granulomatous lesions suggestive of TB with or without presence of langerhans giant cell on histopathology.

ii. **Probable TB –** Those subjects negative on all performed tests but responded to ATT on the clinical- radiological grounds.

iii. Final diagnosis - Combined result of all performed diagnostic tests with ATT responded

Ethics approval: This study was approved by 41st institutional ethics committee "A-04 PGI/IMP/EC/41/28/2/2008".

Statistical analysis:

The final diagnosis was established by using the collective results of ZN microscopy, BACTEC culture and PCR with ATT response. The test tools efficiency was calculated as [(Total number of positive/Total number of analyzed cases] x100 as well as sensitivity [Tp/(Tp + Fn)] x 100 and specificity [Tn/(Tn + Fp)] x 100 were determined. In addition the positive predictive value (PPV) was calculated as [Tp/(Tp + Fn)] x 100, negative predictive value (NPV) was calculated as [Tn/(Tn+Fp)] x 100. (Abbreviations used in above formula - Tp = total number of true positives; Tn = total number of true negative; Fp = total number of false positive, Fn = total number of false negative). The differences in the performed diagnostic modalities were analyzed by the chi - square (χ^2) test. Universal significant p value < 0.05 was taken in this study calculated by SPSS 15.1 version.

RESULTS

Among 55 subjects, 30 (54.5%) males and 25 (45.5%) females (median age was 36 years and ranged from 12 to 78 years) were included in this study. Out of these all included subjects; 24 were new cases having no any previous history of intake ATT and/ or tubercular infection (group A), second; 31 were retreated cases, having a history of intake ATT or came to us with continued taking ATT more than one month included in group B in this study.

Laboratory work up:

We prospectively examined all the specimens on QFT-GIT followed by ZN microscopy, BACTEC culture, PCR and histopathology. The tubercular positive efficiency of the performed tests out of 55 specimens were; 40 (73%) on IGRA, 20 (36.4%) on ZN microscopy, 27 (49%) on BACTEC culture, 37(67.3%) on PCR *IS6110* and 39 (71%) on histopathology. The combined positive result of ZN microscopy and BACTEC culture was 31 (56.4%) showed in this study. Despite, we have ultimately reached to 45 cases diagnosed as definite TB with all performed tests and 10 cases were negative included as a probable TB. On clinical evaluation of these 10 negative cases, they were found to be either partially/incomplete treated or recurrent cases of Pott's disease who responded to modified ATT regimens following their surgery.

The sensitivity of each performed diagnostic test in group A vis a vis group B was described in table 1. In group A, we have established 100% (24/24) diagnosis and in group B, we could detect only 68% (21/31) cases by performed tests carried out ZN microscopy, BACTEC culture, PCR and histopathology. The above diagnostic difference was a statistically significant between groups A vis a vis group B (*p*-value= 0.003). However, individual performance of the ZN microscopy, BACTEC culture and PCR were not shown significant predictive differences between group A and B (*p*-values > 0.05). Whilst, histopathology (*p*-values = 0.02) and QFT-GIT (p-values = 0.03) were showing a significant predictive differences between group A and B showed in Table 1.

Table No. 1: Diagnostic differences between new cases (group-A) and retreated cases (group- B)

Performed test	Group A (n=24)	Group B (n=31)	*P*- value	Total (n=55)
ZN microscopy	10 (42%)	10 (32.2%)	> 0.05	20
BACTEC culture	14 (58.3%)	13(54%)	> 0.05	27
PCR IS6110	19 (79.2%)	18 (58%)	> 0.05	37
Histopathology	21 (87.5%)	18 (58%)	0.02*	39
QFT-GIT	21 (87.5%)	19 (61.3%)	0.03*	40

*Singnificant deference observed

QFT-GIT compared in positive and negative cases on other performed laboratory methods:

Analysis of QFT-GIT results among cases were positive and negative by performed tests showed in table no 2. The sensitivity of QFT-GIT among positive and negative cases on performed conventional bacteriological methods (ZN microscopy + BACTEC culture) was 93.5% and 46%, the significant difference (*p*-value < 0.001) was observed. Again sensitivities of QFT-GIT were 86.5%, 44.4% and 85%, 44% amongst positive and negative cases of PCR and histopathology methods respectively. Further, on the analysis of all 45 definite TB (positive) and 10 probable TB (negative) cases, out of these positive 45 cases, 38 cases detected by QFT-GIT and out of all 10 negative cases, 02 cases detected by QFT-GIT. Hence the final sensitivity of QFT-GIT was 84.4% and 80% specificity. In addition, the QFT-GIT had a 95% PPV and 53.3% NPV (p value < 0.001) respectively.

Table No. 2: Sensitivity of QFT-GIT among positive and negative cases by other performed tests

Performed tests (n=55)	QFT-GIT results (n)		Sensitivity
	No (%)	Pos.	Neg.	QFT-IT
ZN Microscopy positive	20(36.4%)	18	02	90%
ZN Microscopy negative	35(63.6%)	22	13	63%
BACTEC culture positive	27(49%)	25	02	93%
BACTEC culture negative	28(51%)	15	13	54%
(ZN Microscopy with BACTEC culture) positive	31(56.4%)	29	02	93.5%
(ZN Microscopy with BACTEC culture) negative	24(43.6%)	11	10	46%
PCR (*IS6110*) positive	37(67.3%)	32	05	86.5%
PCR (*IS6110*) negative	18(32.7%)	08	10	44.4%
Histopathology positive	39(71%)	33	06	85%
Histopathology negative	16(29%)	07	09	44%
Combined positive results of the above mentioned tests	45(82%)	38	07	84.4%
Combined negative results of the above mentioned tests	10(18%)	02	08	20%

Sensitivity of QFT-IT with other diagnostic modalities:

The sensitivity of QFT-GIT was increased with analysis with combination of performed test in this study. The sensitivity of QFT- GIT were 76.4%, 82%, 84% and 85.4% with combination of conventional bacteriological methods, PCR, histopathology and the combined result of all performed tests respectively.

DISCUSSION

The diagnosis of Pott's disease, usually depend on a combination of clinical, radiological, bacteriological, pathological and molecular tests. While, a definitive TB diagnosis is based on the detection of *M. tuberculosis* on the culture, which usually takes four to six weeks. (19), (20) Clinicians need for other methods, to achieve a rapid diagnosis of active TB and justify the decision to treat the patient. The decision to initiate ATT can be difficult, particularly in Pott's disease where many neoplastic conditions simulate with this disease. For decades, TST has been used as a diagnostic tool to support the physician's decision process (21). The introduction of IGRAs to the clinical laboratories promised with higher sensitivity and specificity in the diagnosis of latent TB (22). The three types IGRAs commercially available: Quntiferon –TB Gold assays (QFN-Gold); Quntiferon-TB Gold *In tube* assays (QFT-GIT); collects limited, Carnegie, Victoria, Australia), and T-STOP. TB assays (Oxford Immunotec, Abingdon, united kingdom) (23), Although IGRAs were primarily developed for the diagnosis of latent TB, clinicians have also been searching for improved diagnostic tools and explored IGRAs for the immune diagnosis of active TB (21). In the present study we have been using the latest version of IGRAs QFT-GIT, it also evaluated for active TB. (23), (24) Most of the studies were performed in low burden setting countries and few studies were done in endemic country like India. (25), (26), (27) Thus, further information is needed in the performance of IGRAs from developing countries where TB is endemic and other factors such as HIV infections is prevalent.

Therefore, the present study was performed at a referral hospital. Here most of tubercular patients came on treatment/relapse or partially treated. In our series out of 55, only 24 patients whom were did not receive any antibiotics or ATT (group A) and 31 patients were came with continuous taking ATT/ relapse cases (group B). Thus, we explored for the first time, the role of the IGRAs in detecting new cases and relapse/partially treated or on ATT among Pott's disease in India.

IGRAs showed the highest positivity among the diagnostic tests used in this study, 40 (73%) on QFT-GIT followed by 39 (71%) on histopathology 37 (67%) on PCR 27 (49%) on BACTEC culture and 20 (36%) cases on ZN microscopy evaluations. We also agree with prior studies have reported IGRAs positivity, Sang et al (28) found 72%, Pai et al (27) found 73% positivity in definite TB cases.

The role of IGRAs in the diagnosis of active TB is less clear particularly endemic country. The meta-analysis suggested that IGRAs have a sensitivity of ~70-90% in active TB. This may lower in high TB incidence setting; its particular reasons were not described by the authors. (29),(30), (31). The overall sensitivity of QFT-GIT in the present study was in 84% (38/45) which is fairly similar to the previous published series. The performance of IGRAs may vary across populations, in relation to background disease prevalence, prevalence of HIV infection, malnutrition, BCG vaccination, exposure to NTM and other factor. (32), (30), (33) The facts of the lower sensitivity of QFT-GIT in the present study, 61.3% positivity found in group B which increased to 87.5% in new cases group A (table1). These positivity differences of group A and B was found to be statistically significant (*p*-value =0. 003). A serial testing of QFT-GIT during treatment of active TB, caused for decreased the sensitivity of assays. (34) Katiyar et al (35) was observed a significant difference in sensitivity of QFT-G assays during chemotherapy of 76 patients with active pulmonary TB.

Our study did not seek to healthy control for the specificity calculation because we evaluate this assay against a diagnostic battery of Pott's disease. In the present study 10 cases were negative by all performed tests. These cases were to be found in group B subjects. They were found to be either partially/incomplete treated or recurrent/refractory cases of Pott's disease. Out of these 10 negative cases, two cases were detected by QFT- GIT, thus 80% specificity was calculated. The specificity of IGRA in our study was low for diagnosis of Pott's disease compared with previously reported data for active pulmonary TB.

(26),(36) This lower specificity could be explained by the fact that IGRAs have not able to discriminate active and latent TB infection (11).

Based on present observations, it is expressed that the QFT-GIT showed higher sensitivity in positive cases on bacteriology/histology or PCR modalities as well as it is also sensitive in negative cases of performed tests. Table.2. It showed higher sensitivity 93.5% with bacteriological confirmed cases and 46% sensitivity in bacteriological negative cases. Meanwhile, QFT-GIT sensitivity was 84.4 % (38/45) in contrast to whom proven by all performed tests and 20% (2/10) more sensitivity in negative cases in this study. In addition, when assays combined with histopathology, the positivity of QFT-GIT was increased to 84% (46/55) followed by 82% (45/55) with PCR, 76.4 % (42/55) with BACTEC culture or ZN microscopy. For early and rapid diagnosis this assay can be applied in combination of ZN microscopy or molecular methods.

CONCLUSIONS

Our results suggest that QFT-GIT more sensitive than conventional and PCR methods. This assay may be useful in the early detection of Pott's disease and it can justify the decision to treat the patient. Until accurate immunological biomarkers are identified, the gold standard for the diagnosis of active tuberculosis continues to be the direct detection, isolation or amplification of *M. tuberculosis* from clinical specimens. PCR can be applied rapidly to identify the bacilli as *M. Tuberculosis* (in contrast to non-tuberculous mycobacteria) and provide useful information on the presence of rifampicin resistance. Moreover, QFT-GIT can be used for as an initial diagnosis test alone or in combination of PCR/ ZN microscopy in the diagnosis of Pott's disease.

ACKNOWLEDGEMENTS

The present study was financially supported by an Intramural project from Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India (PGI/DIR/RC/200/2008). Manoj Kumar gratefully acknowledges the University Grants Commission (UGC), Government of India, New Delhi, for RGNF grant no. F-142 (SC) 2008 (SA III).

REFERENCES

1. Dass B, Puet TA, Watanakunakorn C. Tuberculosis of the spine (Pott's disease) presenting as 'compression fractures'. Spinal Cord. 2002;40:604-8.
2. Sai Kiran NA, Vaishya S, Kale SS, Sharma BS, Mahapatra AK. Surgical results in patients with tuberculosis of the spine and severe lower-extremity motor deficits: a retrospective study of 48 patients. J Neurosurg Spine. 2007;6:320-6.
3. Bailey HL, Gabriel M, Hodgson AR, Shin JS. Tuberculosis of the spine in children. Operative findings and results in one hundred consecutive patients treated by removal of the lesion and anterior grafting. J Bone Joint Surg Am. 1972;54:1633-57.
4. Tuli SM. Treatment of neurological complications in tuberculosis of the spine. J Bone Joint Surg Am. 1969;51:680-92.
5. Singh UB, Bhanu NV, Suresh VN, Arora J, Rana T, Seth P. Utility of polymerase chain reaction in diagnosis of tuberculosis from samples of bone marrow aspirate. Am J Trop Med Hyg. 2006 Nov; 75:960-3.
6. Akcan Y, Tuncer S, Hayran M, Sungur A, Unal S. PCR on disseminated tuberculosis in bone marrow and liver biopsy specimens: correlation to histopathological and clinical diagnosis. Scand J Infect Dis. 1997; 29:271-4.
7. Kolk AH, Kox LF, van Leeuwen J, Kuijper S, Jansen HM. Clinical utility of the polymerase chain reaction in the diagnosis of extrapulmonary tuberculosis. Eur Respir J. 1998;11:1222-6.
8. Dar L, Sharma SK, Bhanu NV, Broor S, Chakraborty M, Pande JN, et al. Diagnosis of pulmonary tuberculosis by polymerase chain reaction for MPB64 gene: an evaluation in a blind study. Indian J Chest Dis Allied Sci. 1998; 40:5-16.
9. Pottumarthy S, Wells VC, Morris AJ. A comparison of seven tests for serological diagnosis of tuberculosis. J Clin Microbiol. 2000;38:2227-31.
10. Feng Y, Diao N, Shao L, Wu J, Zhang S, Jin J, et al. Interferon-gamma release assay performance in pulmonary and extrapulmonary tuberculosis. PLoS One. 2012;7:e32652.
11. Taki-Eddin L, Monem F. Utility of an interferon-gamma release assay as a potential diagnostic aid for active pulmonary tuberculosis. J Infect Dev Ctries. 2012;6:67-72.
12. Ritacco W CS, Palomino JC New developments and perspectives. First Edition ed. In Palomino JC LS, Ritacco V, Editor: Tuberculosis; 2007.
13. Leyten EM, Mulder B, Prins C, Weldingh K, Andersen P, Ottenhoff TH, et al. Use of enzyme-linked immunospot assay with Mycobacterium tuberculosis-specific peptides for diagnosis of recent infection with M. tuberculosis after accidental laboratory exposure. J Clin Microbiol. 2006;44:1197-201.
14. Ananthanarayan R JCP. Mycobacterium-1 tuberculosis. In: Textbook of microbiology. Orient Longman 1996.
15. Siddiqi S. BACTEC 460 TB system, Product and procedure manual. USA: Becton Dickinson Microbiology System, Sparks, MD; 1996.
16. Van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol. 1991;29:2578-86.
17. Eisenach KD, Cave MD, Bates JH, Crawford JT. Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. J Infect Dis. 1990;161:977-81.
18. Bancroft JD SA, Tuner DR. Techniques for Mycobacteria, In: Manual of histological techniques and their diagnostic application. Fourth Ed. Edinburg Churchill Livingstone: Robert Stevenson House; 1996.

19. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. EUR Respir J. 2011;37:100-11.

20. M. Kumar, R. Kumar, A.K. Srivastva, V. L. Nag, A.K.maurya, Sunil G. Babu,. Sensitivity of PCR IS6110 in relation to culture and staining in Pott's disease Indian j of Neurosurgery. 2013;2: (in press).

21. Vesenbeckh SM, Schonfeld N, Mauch H, Bergmann T, Wagner S, Bauer TT, et al. The use of interferon gamma release assays in the diagnosis of active tuberculosis. Tuberc Res Treat. 2012:768723.

22. Bocchino M, Chairadonna P, Matarese A, Bruzzese D, Salvatores M, Tronci M, et al. Limited usefulness of QuantiFERON-TB Gold In-Tube for monitoring anti-tuberculosis therapy. Respir Med. 2010;104:1551-6.

23. Dominguez J, Ruiz-Manzano J, De Souza-Galvao M, Latorre I, Mila C, Blanco S, et al. Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. Clin Vaccine Immunol. 2008;15:168-71.

24. Chee CB, Gan SH, Khinmar KW, Barkham TM, Koh CK, Liang S, et al. Comparison of sensitivities of two commercial gamma interferon release assays for pulmonary tuberculosis. J Clin Micbiol. 2008;46:1935-40.

25. Dogra S, Narang P, Mendiratta DK, Chaturvedi P, Reingold AL, Colford JM, Jr., et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. J Infect. 2007;54:267-76.

26. Kalantri Y, Hemvani N, Chitnis DS. Evaluation of whole blood IFNgamma test using PPD and recombinant antigen challenge for diagnosis of pulmonary and extra-pulmonary tuberculosis. Indian J Exp Biol. 2009;47:463-8.

27. Pai M, Joshi R, Bandyopadhyay M, Narang P, Dogra S, Taksande B, et al. Sensitivity of a whole-blood interferon-gamma assay among patients with pulmonary tuberculosis and variations in T-cell responses during anti-tuberculosis treatment. Infection. 2007;35:98-103.

28. Song KH, Jeon JH, Park WB, Kim SH, Park KU, Kim NJ, et al. Usefulness of the whole-blood interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis. Diagn Microbiol Infect Dis. 2009;63:182-7.

29. Lange C, Pai M, Drobniewski F, Migliori GB. Interferon-gamma release assays for the diagnosis of active tuberculosis: sensible or silly? Eur Respir J. 2009 ;33:1250-3.

30. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med. 2007; 146:340-54.

31. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med. 2008;149:177-84.

32. Adetifa IM, Lugos MD, Hammond A, Jeffries D, Donkor S, Adegbola RA, et al. Comparison of two interferon gamma release assays in the diagnosis of Mycobacterium tuberculosis infection and disease in The Gambia. BMC Infect Dis. 2007;7:122.

33. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess. 2007;11:1-196.

34. Lee SW, Lee CT, Yim JJ. Serial interferon-gamma release assays during treatment of active tuberculosis in young adults. BMC Infect Dis.2010;10:300.

35. Katiyar SK, Sampath A, Bihari S, Mamtani M, Kulkarni H. Use of the QuantiFERON-TB Gold In-Tube test to monitor treatment efficacy in active pulmonary tuberculosis. Int J Tuberc Lung Dis. 2008;12:1146-52.

36. Nishimura T, Hasegawa N, Mori M, Takebayashi T, Harada N, Higuchi K, et al. Accuracy of an interferon-gamma release assay to detect active pulmonary and extra-pulmonary tuberculosis. Int J Tuberc Lung Dis. 2008;12:269-74.

Source of support: University Grants Commission (UGC), Government of India, New Delhi
Conflict of interest: None Declared