Comparative Evaluation of FASTPlaque Assay With PCR and Other Conventional In Vitro Diagnostic Methods for the Early Detection of Pulmonary Tuberculosis

Sarman Singh,^{1*} Taran Prit Saluja,¹ Manjot Kaur,¹ and G.C. Khilnani²

¹*Division of Clinical Microbiology, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India*

²*Division of Chest Medicine, Department of Internal Medicine, All India Institute of Medical Sciences, New Delhi, India*

Rapid and accurate diagnosis of symptomatic patients of pulmonary tuberculosis (TB) is highly desirable to minimize the spread of the disease in the society. We, therefore, compared the usefulness of various conventional diagnostic methods, the in-house polymerase chain reaction (PCR), and the FASTPlaque assay in this study. Laboratory data of 150 patients with clinical diagnosis of pulmonary TB and 50 controls were included in this study. The sputa from all these 200 individuals were subjected to acid-fast staining, culture on Lowenstein–Jensen (L-J) slants, automated BACTEC-MGIT-960 culture methods, and a mycobacteriophage assay. A mycobacterium genus and *Mycobacterium tuberculosis* species-specific PCRs were also done and samples positive on both PCRs were considered as standard for comparison. Of the 5 in vitro diagnostic tests, PCR method was found to be the most rapid, sensitive, and specific, detecting all the 150 cases of pulmonary TB without any false-positive and negative

result. In comparison with PCR the sensitivity of MGIT-960 was 90%, followed by FASTPlaque assay (76.7%), L-J culture method (73.3%), and microscopy (60%). The mean detection time for smear-positive and smear-negative samples was 12.5 and 14 days in MGIT-960 and 18 and 25 days for L-J method, respectively. The FASTPlaque failed to detect mycobacteria from the paucibacillary samples. The contamination rates for MGIT-960, L-J, and FASTPlaque assays were 4, 8 and 10%, respectively. The best correlation with mycobacterial load in the specimen was observed in BACTEC-MGIT-960 showing 66.6% detection rate in paucibacillary, 83.3% in 1+ samples, and 100% in 2+ and 3+ samples. Out of the 150 patients, 140 (93.3%) could be diagnosed by one or more nonmolecular methods. Therefore, it could be concluded that combination of three or more in vitro diagnostic methods will have acceptable detection level. *J. Clin. Lab. Anal.* 22:367–374, 2008. © 2008 Wiley-Liss, Inc.

Key words: tuberculosis; FASTPlaque; PCR; MGIT-960; diagnosis

INTRODUCTION

Tuberculosis (TB), a re-emergent killer disease, is threatening to assume serious proportions all over the world, particularly in view of the AIDS pandemic (1). An essential element in the control of TB is the rapid, sensitive, and specific identification of the causative agent. Until now in most of the developing countries, diagnosis is traditionally made on the basis of clinical symptoms, signs, radiological findings, tuberculin tests, and sputum microscopy, and rarely added with culture for mycobacterium (2). Even in the best clinical setting, diagnosis of TB cannot be relied on symptoms and signs

as several other diseases may manifest in similar manner. Direct microscopic examination of sputum, although insensitive, remains the most rapid screening test, which

Grant sponsor: Department of Biotechnology, Government of India; Grant sponsor: Indian Council of Medical Research, New Delhi.

*Correspondence to: Dr. Sarman Singh, Division of Clinical Microbiology, Department of Laboratory Medicine, All India Institute of Medical Sciences, P.O. Box. 4938, New Delhi 110029, India. E-mail: sarman_singh@yahoo.com

Received 14 April 2008; Accepted 4 June 2008

DOI 10.1002/jcla.20264

Published online in Wiley InterScience (www.interscience.wiley.com).

is also cost-effective. The conventional Lowenstein–Jensen (L-J) culture method is cumbersome, needs long incubation period of 4–6 weeks, and has poor sensitivity (3). Therefore, the mycobacteriology laboratories always aimed at shortening the detection and identification time for mycobacterial infections (4,5).

Recently, time to detection of mycobacteria has been greatly reduced by the use of automated or semi-automated liquid culture systems. The first-generation instruments were based on the use of radiolabeled carbon in the growth medium (6), but more recently nonradiometric systems have also been developed (6,7). Most of the recent systems are fully automated and have further reduced the time to TB diagnosis.

Another upcoming technique, FASTPlaque-TB™ test, utilizes mycobacteriophage to report the presence of viable *Mycobacterium tuberculosis* bacilli in a clinical specimen (8). It is claimed that the technique is most rapid and enables the microbiologist to report within 48 hr whether the specimen had *M. tuberculosis* or not. The test is based upon the principle of phage amplification and utilizes mycobacteriophage as a reporter of cell presence and viability. In this method, the clinical sample containing *M. tuberculosis* is mixed with *M. tuberculosis* species-specific bacteriophage, which is allowed to adsorb and infect the *M. tuberculosis* cells, if present. All unabsorbed extracellular phages are then inactivated, whereas the intracellular phages remain protected and continue to replicate within the *M. tuberculosis* cells. After replication the progeny of bacteriophages lyse the mycobacterial host cell and are released outside. The released bacteriophages, then, are detected by mixing with fast-growing nonpathogenic sensor cells (*M. smegmatis*) on the culture lawn and are counted with naked eyes. The numbers of plaques are direct evidence of the number of viable tubercle bacilli in the sample.

However, the data on this technique are scarce and the technique was evaluated using semi-automated culture systems only where it was reported to be 90.9% sensitive and 88.8% specific (9). We routinely use Ziehl–Neelsen (Z-N) acid-fast stain, L-J, and MGIT-960 culture systems for our diagnostic services. Recently, a 14 kDa early secreted antigenic target (ESAT) protein of *M. tuberculosis* has been reported to be highly species specific for *M. tuberculosis* complex species (10–13) and in our laboratory we have developed a novel set of polymerase chain reaction (PCR) primers targeting the ESAT-6 gene (14). The utility of ESAT-6 PCR has been published elsewhere (14), and the product and the process are patented. In the present communication, utility of FASTPlaque-TB™ test was compared with our in-house PCR, MGIT-960 automated culture system, and other conventional detection methods.

MATERIALS AND METHODS

Patients and Samples

In this study, which was approved by the institutional ethical committee, we included 150 patients referred from chest clinic of this hospital with presumptive clinical diagnosis of pulmonary TB. Fifty patients attending the medical outpatients department for acute upper respiratory tract infection with no chest signs and clear X-ray pictures comprised the control group. Patients detected seropositive for HIV, and patients on antitubercular treatment for more than 7 days were not included in these data. Also all samples that were genus-specific PCR positive but *M. tuberculosis* species-specific PCR negative were not included in this study. The isolation rate and other details of various nontubercular mycobacteria in our laboratory are published elsewhere (14).

Specimen Processing

From all patients three consecutive samples were obtained and stored at +4°C till further use. All the three sputa from a patient were pooled, mixed, digested, and decontaminated following the standard protocol (15). In brief, about 5–6 ml of contaminated specimen was mixed with an equal volume of 0.5% NALC–4% NaOH mixture in 50 ml ridge capped round bottom processing tube; the mixture was vortexed and incubated at 37°C for 10 min. After incubation, mixture was neutralized with phosphate buffer (pH 6.8) up to a total volume of 50 ml followed by centrifugation at 8,000 × *g* for 10 min. The supernatant was decanted and the pellet was resuspended in 2 ml of the phosphate buffer saline. From the decontaminated sample, 0.5 ml of the suspension was inoculated into the BACTEC™-MGIT tubes, 0.1 ml in L-J slants under sterile conditions in the biosafety cabinet type 2 (Kartos, India). From a portion of this suspension smears were prepared and stained with Z-N acid-fast staining (15). The smears were also prepared directly from the unprocessed samples simultaneously as a routine patient-care service. The mycobacterial load (semi-quantitative) was estimated from decontaminated smears according to the World Health Organization (WHO) method.

Culture in L-J and MGIT-960 Systems

A portion of the specimen, prepared as described above, was inoculated onto the L-J culture slants (0.2 ml) and MGIT-960 (Becton Dickinson®, Sparks, MD) culture broth tubes (0.5 ml). The L-J slants were incubated at 37°C for up to 6 weeks. Prior to the inoculation of the MGIT-960 tube, oleic acid–albumin–dextrose–citric acid (16) growth supplement and

antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) were added to it. The tubes were incubated at 37°C in the BACTECTM-MGIT-960 instrument and were monitored automatically every 60 min for increase of fluorescence (16). A series of algorithms included in the instrument is used to determine the presumptive positivity and to sound an alarm for the presence and locations of the positive tubes. The sample was considered negative if no growth was obtained in L-J slants after 6 weeks of incubation and 42 days in the MGIT-960 system. Any sample, which was identified by the instrument as positive, was removed from the tube station, and smears were prepared and stained with Z-N and Gram's stains to confirm the growth.

FASTPlaque-TB Assay

This assay was performed using FASTPlaque-TBTM kit (Biotech Laboratories, Ipswich, UK). The samples were processed according to the manufacturer's instructions (8). Positive and negative controls, containing 1 ml suspension of *M. smegmatis* (about 10⁵ organisms) in broth and broth without *M. smegmatis*, respectively, were included in all experiments. One milliliter of the decontaminated and concentrated suspension of the sample, as described above, was mixed with 1 ml of FASTPlaque-TBTM broth and incubated at 37°C overnight to enrich the viable tubercular bacilli, if present in the sample. Next morning 100 µl of mycobacteriophage solution was added to the sample suspension and incubated for further 1 hr to allow the bacteriophage infection of the tubercular bacilli. Extracellular phages were neutralized by adding 100 µl of virucide (provided with the kit) to each sample, followed by thorough mixing and incubation at room temperature for 5 min. Then 5 ml FASTPlaque-TB medium was added to neutralize excess virucide, followed by 1 ml of

sensor cells and 5 ml of molten agar, mixed thoroughly, and poured onto the sterile petri dish. On pouring, plates were rotated several times, both clockwise and counterclockwise, to facilitate the mixing of phage-infected cells and *M. smegmatis* into the agar. Plates were allowed to set, incubated for 18 hr at 37°C, and the number of plaques was read. When the clearing of *M. smegmatis* growth was insufficiently dense to allow the visualization, the incubation period was extended to 24 hr. In negative results no plaque was seen, whereas samples with more than 25 plaques were considered as positive. Samples with plaques between 20 and 25 were considered as indeterminate. For such samples repeat test was done. Samples with high mycobacterial units showed confluent clearing of the growth (plaques) of *M. smegmatis* (Fig. 1, Panels A, B, and C, respectively).

Sample Preparation and Protocol for PCR

Mycobacterial DNA from the decontaminated clinical samples (see above) was extracted using the standard protocol described elsewhere (17). As sputum might have high concentration of PCR inhibitors, these substances were removed using 10% chelex 100 resin (Sigma, St. Louis, MO) in the lysis buffer.

All samples were subjected to two PCRs: the first one genus-specific PCR in which internal transcribed spacer (ITS) region of 16–23 S rRNA primers were used (18). These primers named as ITS-F and Mycom-2 are designed from the highly conserved region in all mycobacteria. The primer sequences were:

ITSF: 5' tgg atc cga agt cgt aac aag g 3' and

Mycom-2: 5' atg ctc gca acc act atc ca 3'.

The PCR was run in a 50 µl mix follows: 500 mM KCl, 100 mM Tris HCl (pH 9.0), 1% Triton X-100, 0.2 mM each deoxynucleoside triphosphate (dATP, dGTP, dTTP, and dCTP), 1.5 mM MgCl₂, 10 pmol of each

Fig. 1. FASTPlaque-TBTM method for identification of the *Mycobacterium tuberculosis*. All culture plates are seeded with *M. smegmatis* as substrate (sensor cells) for mycobacteriophage. Panel A: FASTPlaque-TBTM. Negative control, showing no lysis (plaque formation) of the sensor cells. Panel B: FASTPlaque-TBTM. Positive control, showing more than 20 plaques (virucidal units). Panel C: FASTPlaque-TBTM. Showing heavy mycobacterial load in the clinical samples, indicated by presence of confluent (>200) plaque formation.

primer, and 1 U of *Taq* DNA polymerase (Promega, Madison, WI). Each reaction was carried out for 5 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C; and 10 min at 72°C. The products were electrophoresed in 1.5% agarose gels. The amplicon sizes varied between 270 and 400 bp in size depending on the species of mycobacterium. The PCR system was standardized on pure growth of standard mycobacterial strains and sputum samples from patients having bacteriologically confirmed *M. tuberculosis* and nontubercular mycobacterial infections (Fig. 2, Panels A and B).

For *M. tuberculosis* species-specific PCR, primers designed to amplify full-length *esat-6* (Rv3875) gene (AF420491) were used.

The primers sequences are:

ESAT-6F: 5' gcg gat ccc atg aca gag cag cag tgg a 3' and

ESAT-6R: 5' ccc aag ctt cct atg cga aca tcc cag tga cg 3'.

The constituents for species-specific PCR mixtures were same as that for the genus-specific PCR. The PCR reaction cycle conditions included initial denaturation at 94°C for 5 min; followed by 30 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C; followed by final extension at 72°C for 10 min. The products were electrophoresed in 1.5% agarose gels to resolve the PCR product of 320 bp (Fig. 2, Panel C).

RESULTS

The age of the study group patients ranged from 10 to 65 years (mean $37.5\% \pm 13.10$). Of these 95 (65.3%) were males with mean age of 32 ± 10.74 years and 55 (33.6%) were female patients with mean age of

Fig. 2. Panel A: Lane M, 100 bp Marker, Lanes 1,5,7, *M. tuberculosis*; Lane 2, *M. phlei*; Lane 3, *M. smegmatis*; Lane 4, *Mycobacterium duvalii*; Lane 6, *Mycobacterium bovis* BCG; Lanes 8 & 9, *M. avium*, Lane 10, *M. austroafricanum*, Lane 11, *M. celatum*; and Lane 12, *M. vaccae* (Amplicon sizes between of 270–400 bp depending on the Mycobacterial species). Panel B: PCR amplification of 270–400 bp of 16-23SrRNA using genus specific primers ITS-F and Mycom-2; Lane M, 100 bp Marker; lanes 1–10, PCR using DNA from clinical samples; Lane 11, positive control (DNA from standard H37Rv); Lane 12, negative control (sterile water). Panel C: PCR amplification of 320 bp *esat-6* region which is *M. tuberculosis* complex specific. The Lane 1, 100 bp Marker; lanes 1–9, PCR using DNA from *M. tuberculosis* culture proven clinical samples.

29 ± 11.55. In the control group, 31 (62%) were males and 19 (38%) females. The mean age of controls was 34 ± 13.7. The cough was invariably seen in all (100%) patients followed by fever (93.3%), loss of weight (60%), and hemoptysis (13.3%).

Smears from all the sputa were prepared after concentration (see "Material and Methods") followed by heat fixation and acid-fast staining as per standard Petroff's procedure (15). Samples from all 150 (100%) patients were PCR positive and MGIT-960 recovered mycobacteria from 135 (90%) patients. The L-J culture had detection rate of 73.34%, whereas 90 (60%) patients were found positive for acid-fast bacilli in smear microscopy. The FASTPlaque-TB™ assay was only marginally better than L-J culture and smear microscopy with detection rate of 76.7% (115/150) (Table 1).

Significant observations were made in respect to the growth in L-J and MGIT cultures and the quantification of acid-fast bacilli seen on the smear examination. The culture medium and bacterial load influenced the initial time of detection of mycobacteria in all the growth systems including the FASTPlaque TB™ assay. It was observed that the greater the bacterial load on microscopy, the shorter the average time taken for detection in both the culture systems. Positive growth in MGIT-960 system was detected within 7–14 days in 135 (90%) cases with the mean time of 12.5 days from smear-positive cases but 14 days in smear-negative samples (Table 2).

The main aim of this study was to compare the diagnostic utility of various systems in comparison with the mycobacterial load in the sputum sample. The mycobacterial load was estimated using a semi-quantitative method using the WHO criteria, in which samples were graded on the scale of 1+ to 3+ positivity (2). In FASTPlaque-TB™ system, none of the smear-negative samples was found positive. Overall 120 (80%) PCR-positive samples were also smear positive, whereas both

MGIT-960 and L-J cultures could detect 20 of 30 (66.6%) samples as positive. Of the 30 smear-positive (+) samples, 10 (33.3%) showed confluent lysis (>200 plaques) (Fig. 1, Panel C), 25 (83.3%) of these were detected positive by MGIT-960 system between 15 and 22 days, and 5 remained negative even after 42 days of incubation. L-J cultures could also detect *M. tuberculosis* from 20 (66.6%) samples. Out of 70 smear-positive (2+) samples, a total of 65 (92.8%) were FASTPlaque positive but 50 (71.4%) showed confluent lysis. All the 50 samples with confluent growth (100%) were also detected by L-J culture method, but MGIT-960 could detect all the 70 samples (Table 2). All of the 20 smear-positive (3+) samples were correctly detected by all the systems.

The average detection time by the L-J culture method was 18 (15–45) days for smear-positive and 25 (20–45) days in smear-negative samples. Overall 140 (93.3%) samples were positive by more than one diagnostic test in addition to the PCR. Only ten PCR positive samples were negative in all the microscopy, MGIT-960, L-J culture, and FASTPlaque-TB™ assay methods, indicating that no conventional method can accomplish 100% sensitivity (Table 2). This finding also emphasizes the need for molecular methods in routine diagnostic laboratories, which are rapid, most sensitive, and cost-effective also.

Contamination rate was highest in FASTPlaque-TB™ assay (10%) followed by L-J culture (8%) and MGIT-960 (4%). Although on FASTPlaque-TB™ plates molds were commonest contaminants, in L-J and MGIT-960 bottles bacterial contaminants were seen. No other test method showed false-positive results except FASTPlaque-TB™ assay. The FASTPlaque-TB™ assay showed false-positive results in 7 (14%) samples, indicating a specificity rate of 86%. Inter-observer evaluation of FASTPlaque-TB™ assay was not very appreciated. In plates with complete clearing of growth indicating confluent growth, some observers got confused as no growth, which required more training. Therefore, the sensitivity of FASTPlaque-TB™ was not better than microscopic examination of Z-N-stained smears, whereas its specificity was 86%.

TABLE 1. Sensitivity and Specificity of Z-N Smear, L-J Culture, MGIT-960 Culture, and FASTPlaque-TB™ Assay in Comparison With PCR

Patient group	Smear positive	L-J culture positive	MGIT-960 culture positive	FASTPlaque assay positive
Study (n = 150)	120	110	135	115
	80%	73.3%	90%	76.6%
Control (n = 50)	0	0	0	7
	0	0	0	14%
Total	120	110	135	122
Sensitivity (%)	80	73.3	90	76.6
Specificity (%)	100	100	100	86

Z-N, Ziehl-Neelsen; L-J, Lowenstein-Jensen; PCR, polymerase chain reaction.

DISCUSSION

Pulmonary TB is a major killer disease in India and accounts for more than 25% of the avoidable deaths worldwide. India ranks as a highest TB burdened country. The current epidemic of HIV/AIDS has further made the situation worst. Like HIV/AIDS, TB also affects the most productive age group of males. Though the number of cases in this study was less, the male to female ratio was in concordance with the well-documented disease pattern in India (19). In this study our

TABLE 2. Meantime for Detection and Mycobacterial Load in the FASTPlaque TB™ Assay and MGIT-960 System in Relation to Smear Grading

Z-N smear	L-J culture		FASTPlaque assay				MGIT-960				
			Number of plaques				Period to detect positive (d)				
WHO grading	Pos.	NG	<20	25–50	50–200	>200	7–10	11–14	15–18	19–22	NG
Negative (30)	20	10	30	–	–	–	–	–	10	10	10
+	20	10	–	10	10	10	–	–	10	15	5
++ (70)	50	20	5	5	10	50	5	30	25	10	–
+++ (20)	20	0	–	5	–	15	10	10	–	–	–
Total 150	110	40	35	20	20	75	15	40	45	35	15

Z-N, Ziehl–Neelsen; L-J, Lowenstein–Jensen; WHO, World Health Organization; Pos., positive; NG, no growth. The assay with plaque number between 20 and 25 was not included in any category and the final result was considered only after repeat testing, which showed either lower than 20 plaques or more than 25 plaques.

male patients outnumbered the females. Cough was the most common symptom of pulmonary TB, and it was also the key to clinical suspicion of pulmonary TB (20). As all our patients were selected from the specialized chest clinic with high index of clinical suspicion of pulmonary TB, it is not surprising that all of our patients had this most important clinical finding and that all were PCR positive. However, these clinical symptoms may not be very useful in early stage of the disease. Even in India where TB is highly endemic, diagnosis of active pulmonary TB can be a diagnostic dilemma (20).

There have been several investigations of the individual usefulness of various laboratory methods for rapid and accurate diagnosis of TB. The most rapid, cost-effective, and most commonly used technique in most of the resource-poor countries is acid-fast staining. This method is also being used successfully in the WHO's directly observed treatment short course program in India (21). In addition, because it assesses the infectivity of patients with pulmonary TB, it is an indispensable test for hospital and community infection control (22). But the inherent problem of acid-fast staining method remains to be its low sensitivity and inability to distinguish between the dead and live bacilli (22). In this study, the 60% sensitivity of the smear examination as compared with PCR may seem to be very high, but we would not like to make conclusion from this study, because the cases in this study were selected from the chest clinic with a very high clinical index of pulmonary TB. Patients with no supportive radiological and other surrogate investigations were not included in this study, as the objective of this study was only to compare the detection rate of various non-PCR-based diagnostic assays. To study the phenotypic or genotypic characteristics and antibiotic susceptibility, the bacteria must be cultured *in vitro*. Most commonly used culture method is L-J medium. But the L-J culture method is very time

consuming, tedious, and carries a low detection rate (22). In this study also, it showed lower detection rate (73.3%) than acid-fast stain; the other diagnostic methods were more superior with better detection rate as reported by others too (23,24). There are several reasons for poor detection rate by L-J culture method, which include batch to batch variation in the eggs used in the medium, as well as neutralization of the strong alkali used in the decontamination process.

The molecular methods have gained upper hand over all the conventional laboratory methods (4). In this study both our genus-specific and species-specific PCR methods showed excellent sensitivity and specificity, without missing any case; hence, PCR was used to compare the results of other methods. While comparing the sensitivity of other diagnostic methods with PCR technique, we found that MGIT-960 had highest (90%) recovery rate for *M. tuberculosis*. It was also true for other mycobacterial species but these results are not included here and have already been published elsewhere (14). However, in our setup that has high environmental fluctuations, this system also showed contamination rate to the tune of 4% (2–10%, depending on the season). Most of the times it was bacterial contamination, but at occasions the system gave positive signal but Gram-stained smears failed to demonstrate any bacteria or fungus. As the system is based on sensing the concentration of carbon dioxide in the medium, it is possible that fluorescence sensors get falsely stimulated owing to electricity and temperature fluctuations. However, the average contamination rate was not significantly different from other western laboratories (6,24). As expected the utility of L-J culture method was less rewarding in comparison with automated culture technique and PCR but not as dismal as reported by Mejia et al. (25). These authors found overall recovery rates of only 11%. Even though we had recovery rate of 60%, we strongly feel that this

conventional method may not lose its importance very soon. It is a time-tested technique and is still being used as the benchmark for mycobacterial disease diagnosis, particularly in resource-poor laboratories.

Our main purpose in this study was the evaluation of FASTPlaque assayTM system vis-à-vis what we are already using in our laboratory. This system has been launched with high enthusiasm, which is claimed to specifically diagnose the *M. tuberculosis* infection within 48 hr after the receipt of clinical sample (8). The manufacturers propose that the test is 100% sensitive and can be performed at a class-I biosafe mycobacteriology laboratory. In this study we found this method to be 76.6% sensitive in comparison with PCR method and 85.1% in comparison with MGIT-960. It had only marginally better sensitivity than L-J culture method (Table 2). However, the method also had false-positivity rate of 14% and the contaminants were invariably molds. The high contamination rate can be explained on the basis of richness of its medium and surface area of the culture plate used. Also the culture medium for preparing the lawn of *M. smegmatis* takes a few minutes and the time and the process involved in transferring the warm medium from flask to petri plate are probably the culprits. Probably during this process the circulating molds get into the medium. The 10% contamination rate was highest for any diagnostic method and this may limit its utility at level-I biosafety TB laboratories in the developing world with hot and humid climatic conditions.

Another problem was false-positive or indeterminate results. This was because the cut-off number of plaques given in the assay protocol for the negative sample is 20 plaques and for positive results more than 25. That means that the indeterminate samples had to be repeat tested and we found that this was not reproducible. Also its utility was better than smear microscopy in paucibacillary samples. Moreover, the number of plaques did not correlate with the bacterial load in the samples (Table 2).

In conclusion, the FASTPlaque assayTM system was not better than smear microscopy with inherent low detection rate for paucibacillary TB, added with high false-positivity and contamination rates. The cost of FASTPlaque assay system was marginally lower but not so attractive and significantly less than PCR.

ACKNOWLEDGMENTS

The authors thank Mr. Bhupendra Kumar and Mr. Nand Kishore for their technical help. We also thank the Medispan Ltd., Secunderabad, India, for partial free supply of the FASTPlaque assay kits for this study. The partial financial support provided to carry out this study

by the Department of Biotechnology, Government of India, and the Indian Council of Medical Research, New Delhi (to S.S.) is also acknowledged.

REFERENCES

- Centers for Disease Control and Prevention (CDC). Progress toward tuberculosis control—India 2001. *Morb Mortal Wkly Rep* 2002;51:229–232.
- World Health Organization. 1998. Part II. Microscopy. Laboratory Services in Tuberculosis Control. Geneva, Switzerland: World Health Organization. 43 p.
- Sharp SE, Lemes M, Sierra SG, Poniecka A, Poppiti RJ, Lowenstein-Jensen media. No longer necessary for mycobacterial isolation. *Am J Clin Pathol* 2000;113:770–773.
- Bennedsen J, Thomsen VO, Pfyffer GE, et al. Utility of PCR in diagnosing pulmonary tuberculosis. *J Clin Microbiol* 1996;34:1407–1411.
- World Health Organization. 1998. Part III. Culture. Laboratory Services in Tuberculosis Control. Geneva, Switzerland: World Health Organization. 77 p.
- Lorenz L, Sören S, Bettina B, Adelheid M, Jürgen H, Roggenkamp A. Evaluation of BACTEC MGIT 960 and BACTEC 460TB systems for recovery of mycobacteria from clinical specimens of a university hospital with low incidence of tuberculosis. *J Clin Microbiol* 2001;39:3764–3767.
- Tortoli E, Mattei R, Savarino A, Bartolini L, Beer J. Comparison of *Mycobacterium tuberculosis* susceptibility testing performed with BACTEC 460TB (Becton Dickinson) and MB/BacT (Organon Teknika) systems. *Diagn Microbiol Infect Dis* 2000;38:83–86.
- Biotech FAST Plaque-TBTM. An innovative breakthrough for the detection of tuberculosis. Technical data report, BIOTECH Laboratory Ltd., UK, 2000.
- Shenai S, Rodrigues C, Mehta AP. Evaluation of new phage amplification technology for rapid diagnosis of tuberculosis. *Indian J Med Microbiol* 2002;20:194–199.
- Pollock JM, Andersen PA. The potential of the ESAT-6 antigen secreted by virulent mycobacteria for specific diagnosis of tuberculosis. *J Infect Dis* 1997;175:1251–1254.
- Andersen PA, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;356:1099–1104.
- Ravn P, Demissie A, Eguale T, et al. Human T cell responses to the ESAT-6 antigen from *Mycobacterium tuberculosis*. *J Infect Dis* 1999;179:637–645.
- Van Pinxteren LAH, Ravn P, Agger EM, Pollock JM, Anderson P. Diagnosis of tuberculosis based on the two specific antigen ESAT-6 and CFP 10. *Clin Diagn Lab Immunol* 2000;7:155–160.
- Singh S, Gopinath K, Shahdad S, Singh B, Sharma P. Nontuberculous mycobacterial infections in Indian AIDS patients detected by a novel set of ESAT-6 polymerase chain reaction primers. *Jpn J Infect Dis* 2007;60:14–18.
- Kent PT, Kubica GP. 1985. Public Health Mycobacteriology. A Guide for Level III Laboratory, Vol. 1. Atlanta, GA: Center for Disease Control.
- Kanchana M, Cheke VD, Natyshak I, Connor B, Warner A, Martin T. Evaluation of the BACTEC MGIT 960 system for the recovery of mycobacteria. *Diagn Microbiol Infect Dis* 2000;37:31–36.
- Chakravorty S, Tyagi S. Novel use of guanidinium isothiocyanate in the isolation of *Mycobacterium tuberculosis* DNA from clinical material. *FEMS Microbiol Lett* 2001;205:113–117.

18. Park H, Jang H, Kim C, et al. Detection and identification of mycobacteria by amplification of internal transcribed spacer region with genus and species specific PCR primers. *J Clin Microbiol* 2000;38:4080–4085.
19. Mohan A, Sharma SK. 2001. Tuberculosis epidemiology. In: A Mohan, SK Sharma, editors. *Tuberculosis*. New Delhi: Jaypee Brothers Medical Publishers. p 14–29.
20. Anonymous. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000;161:1376–1395.
21. Khatri GR, Frieden TR. The status and prospects of tuberculosis control in India. *Int J Tuberc Lung Dis* 2000;4: 193–200.
22. Watterson SA, Drobniewski FA. Modern laboratory diagnosis of mycobacterial infections. *J Clin Pathol* 2000;53:727–732.
23. Hanna BA, Ebrahimzadeh A, Elliott LB, et al. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. *J Clin Microbiol* 1999;37:748–752.
24. Somoskovi A, Magyar P. Comparison of mycobacterial growth indicator tube with MB redox, LJ and middlebrook 7H11 media for recovery of mycobacterium in clinical specimens. *J Clin Microbiol* 1999;37:1366–1369.
25. Mejia GI, Castrillon L, Trujillo H, Robledo JA. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of *Mycobacterium tuberculosis* infection. *Int J Tuberc Lung Dis* 1999;3:138–142.