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Original Article

Effectiveness of the BDProbeTec ET system for detection of *Mycobacterium tuberculosis* complex in sputum and bronchoalveolar lavage specimens

Hung-Chang Hung[a,b,c], *Chi-Ho Chan*[b,d], *Shih-Ming Tsao*[a,b,e,f], *Shiuan-Chih Chen*[a,b,g], *Tzu-Chin Wu*[a,b,e], *Min-Chi Lu*[a,b,f], *Der-Jinn Wu*[a,b,h], *Shu-Ju Huang*[a,b,f], *Yuan-Ti Lee*[a,b,f,*]

[a]*Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[b]*Faculty of Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[c]*Potz Hospital, Department of Health, Executive Yuan, Chiayi, Taiwan*
[d]*Department of Microbiology and Immunology, Chung Shan Medical University, Taichung, Taiwan*
[e]*Division of Chest, Department of Internal Medicine, Chung Shan Medical University, Taichung, Taiwan*
[f]*Division of Infectious Diseases, Department of Internal Medicine, Chung Shan Medical University, Taichung, Taiwan*
[g]*Department of Family and Community Medicine, Chung Shan Medical University, Taichung, Taiwan*
[h]*Division of Cardiology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan*

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ABSTRACT

Objective: The diagnostic efficacy of the BDProbeTEC ET *Mycobacterium tuberculosis* (MTB) complex direct detection assay (DTB) performed on bronchoalveolar lavage (BAL) specimens and sputum smears was compared with acid-fast bacilli (AFB) smear microscopy.
Method: AFB smear microscopy, DTB and culture results of 286 patients with pulmonary tuberculosis were retrospectively reviewed. A total of 120 patients provided expectorated sputum samples, and 166 patients provided BAL specimens. Culture results and clinical diagnosis were used as gold standards.
Results: The sensitivity and specificity of the DTB assay in detecting MTB in sputum specimens was significantly higher compared to AFB smear microscopy (83.7% and 82.4%, vs. 75.6%, and 41.2%, respectively). The sensitivity and specificity of the DTB assay in detecting MTB in sputum samples was 77.2% and 100% compared to clinical diagnosis, while AFB smear had a sensitivity and specificity of 70.3% and 26.3%, respectively. Compared to culture, DTB had a sensitivity and specificity of 82.8% and 93.2%, respectively, in detecting MTB from BAL specimens; AFB smear had a sensitivity and specificity of 41.9% and 87.7%, respectively. Compared to clinical diagnosis, DTB had a sensitivity and specificity of 67.2% and 100%, respectively, in detecting MTB from BAL specimens; AFB smear had a sensitivity and specificity of 34.8% and 79.5%, respectively.
Conclusions: The superior performance of the DTB assay relative to AFB smear microscopy makes it a valuable tool to enable early diagnosis of MTB, thereby improving patient care and reducing transmission.

*Corresponding author at: Division of Infectious Diseases, Department of Internal Medicine, Chung Shan Medical University Hospital, No.110, Sec.1, Jianguo N. Rd., South District, Taichung City 40201, Taiwan
E-mail address: leey521@yahoo.com.tw (Yuan-Ti Lee)

Introduction

Tuberculosis (TB), one of the most serious infectious diseases in the world, is responsible for approximately 2 million deaths every year.[1] The incidence of TB has been reported to be almost 75 per 100,000,[2] and the rate of mortality is about six per 100,000 in Taiwan.[3] Almost 9% of the TB cases worldwide have been reported to occur simultaneously with human immunodeficiency virus (HIV), and one of the leading causes of death in HIV patients is due to opportunistic tuberculosis infection.[4] Approximately 8.8% of new patients with pulmonary tuberculosis in Taiwan have a delayed diagnosis,[5] resulting in more extensive disease, more complications, and higher mortality. In addition, delayed treatment increases the risk of transmission.[6] Therefore, rapid detection, accurate diagnostic methods, and appropriate treatment are essential to control successfully TB.

The gold standard for diagnosing *Mycobacterium tuberculosis* (MTB) is culturing the bacilli from patient specimens.[7] At present, the diagnosis of TB is most commonly made by direct acid-fast bacilli (AFB) smear examination and mycobacterial culture, which often takes three to six weeks. AFB smear microscopy, although rapid and inexpensive, has low sensitivity and specificity.[8] In clinical laboratories in Taiwan, the typical sensitivity of AFB smear microscopy is 42.3%,[9] providing at best a preliminary diagnosis. Ziehl-Neelsen staining, auramine O rhodamine, and immunostaining using polyclonal anti-*Mycobacterium bovis* were all shown to be more efficient than bacteriology.[10] Diagnostic yield of MTB in bronchoalveolar lavage (BAL) was far superior to other clinical specimens such as bronchial brushings, BAL fluid, transbronchial lung biopsy, and bronchial aspiration sputum, and although acquisition of these specimens is an invasive procedure, no complications or infectious events were reported.[11] Bronchoscopy samples provided high rates of detection of MTB (87%) even in patients whose AFB smear samples were negative after testing with high-sensitivity methods, such aspolymerase chain reaction (PCR),[12] and it was shown that 40% of these cases would have been found negative if bronchoscopy had not been performed.

Becton Dickinson (Sparks, MD, USA) has developed and commercialized a semiautomated system, the BDProbeTec ET direct detection assay (DTB), for the rapid detection of MTB in respiratory specimens.[13,14] The DTB assay, which does not use radioisotopes or require experienced operators, is a sensitive (82.7% to 95.8%) and specific (> 90%) diagnostic test for pulmonary TB in adults.[9,13-18] The DTB assay has a slightly lower sensitivity in extrapulmonary samples.[17,19] However, only two investigations have evaluated the DTB assay in the context of a standard clinical laboratory in Taiwan,[18,20] and there is no comparison study on the effectiveness of the DTB assay and AFB smear microscopy in detecting MTB in patient specimens in Taiwan.

The objective of this study was to evaluate the effectiveness of the DTB assay, compared to AFB smear microscopy, in detecting MTB in expectorated sputum samples or BAL specimens, using either culture or clinical diagnosis as the reference standard in a clinical setting characterized by high prevalence of TB.

Materials and methods

Patients

The charts of 786 patients who presented at the Division of Infectious Diseases, at the Division of Chest, or at the Department of Internal Medicine of the Chung Shan Medical University Hospital (CSMUH) with symptoms of lung infection (pulmonary and extra-pulmonary symptoms) between August 2006 and July 2009 were retrospectively reviewed. This study was approved by the Institutional Review Board of the CSMUH (No. CS05123).

Of the 786 patients, 151 patients were excluded because of loss to follow-up, referral to other hospitals, and missing data; 53 patients were excluded because of extrapulmonary TB. Of the 582 patients with pulmonary TB, 416 patients provided expectorated sputum samples, and 166 provided BAL samples, while 296 of the 416 patients were excluded due to lack of DTB data. Therefore, only 120 (416 – 296 = 120) patients who provided expectorated sputum samples and 166 patients who provide BAL samples were included in the final analysis.

The DTB assay was directly compared with AFB smear microscopy and culture/clinical diagnosis in the 120 patients who provided expectorated sputum samples, and in the 166 patients who provided BAL specimens. Fig. 1 is a schematic representation of the study and shows the categorization of the patients into the different diagnostic groups.

Collection of sputum specimens

Patients suspected to have active pulmonary TB were instructed to collect spontaneous expectorated sputum samples at the hospital. Sputum samples were stored at 20°C to 80°C for no longer than 48 hours before the assay. The sputum specimens were added to an equal volume of NaOH-citrate-N-acetyl-L-cysteine for decontamination and digestion prior to culture and DTB assay.

Collection of BAL specimens

The indications for bronchoscopy to collect BAL specimens were: 1) when clinical symptoms and signs suggested TB, 2) when sputum specimens could not be obtained, or 3) when a lung mass was present (to differentiate TB from malignancy). BAL specimens were collected as described by Kobashi et al.[11] during examination with a fiberoptic bronchoscope (model BF20 or P20, Olympus –Tokyo, Japan). The procedure was performed in a negative pressure room, and the clinicians used personal protective equipment during the examination. Patients undergoing this procedure signed an informed consent before bronchoscopy.

Diagnostic testing

Specimens for AFB smear microscopy were processed within 24 hours after collection as previously described.[18] Specimens that could not be processed upon receipt were stored at 2°C to 8°C for no longer than 48 hours. Culture was performed

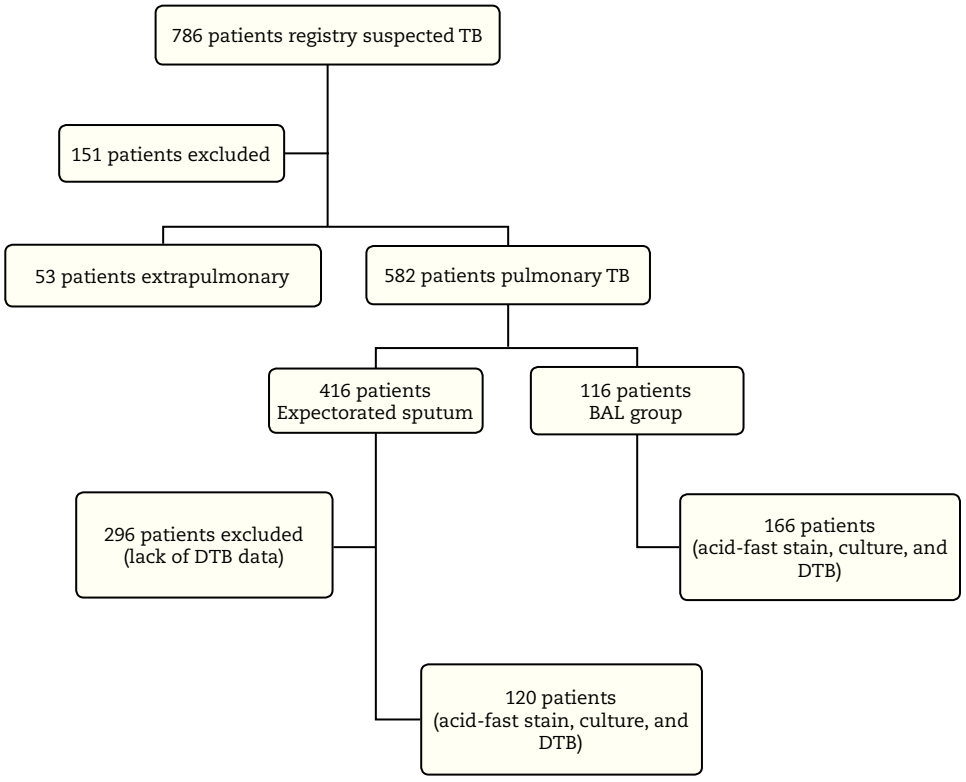

Fig. 1 - Flowchart for the categorization of patients into different diagnostic groups. TB, tuberculosis; BAL, bronchoalveolar lavage; DTB, direct detection assay.

with the BACTEC Mycobacteria Growth Indicator Tube 960 system (Becton-Dickinson Diagnostic Instrument Systems – Sparks, MD, USA) as described previously.[18] All liquid medium cultures were tested until they showed positive results, or for 42 days.[9,16,18] All specimens were inoculated onto conventional solid L-J medium. An individual plate was considered positive if colonies appeared on the surface. Identification of MTB was performed based on standard physical and biochemical characteristics, and was ultimately confirmed by AFB smear microscopy.

BD ProbeTec ET (DTB system)

The DTB assay was performed according to the manufacturer's instructions (Becton Dickinson) and as described previously.[9,13-18] Briefly, 500 µL of decontaminated specimen were added to 1.0 mL of wash buffer, vortexed, and centrifuged. The pellet was heated in a self-contained oven to render the bacteria non-viable. The pellet was then resuspended in lysis buffer, mixed, placed in a sonic water bath, centrifuged again, and the pellet was resuspended in sample neutralization buffer. The mixture was either vortexed, centrifuged, and tested, or frozen at -20°C. Frozen specimens were thawed, heated, centrifuged, and then processed as described for fresh samples.

Samples and controls (150 µL; one positive and three negative controls per run) were dispensed into priming microwells, incubated at room temperature for at least 20 minutes, and then heated at 72.5°C for 10 minutes. Samples (100 µL from each priming microwell) were then transferred into an amplification microwell of a preheated amplification microwell plate. The wells were sealed and the plate was immediately placed into the BDProbeTec ET instrument. After one hour, metric other than acceleration (MOTA, a measurement of the area under the relative fluorescent unit curve) values were noted for each sample and control. Samples with MOTA values greater than 3,400 were considered positive for MTB complex deoxyribonucleic acid (DNA). If the MTB MOTA was < 3,400 and the internal amplification control MOTA was > 5000, then the sample result was considered negative. Samples with MTB MOTA values < 3,400 and internal amplification control values < 5,000 were considered to contain inhibitors of amplification.

Clinical diagnosis of tuberculosis

Medical records were reviewed for evidence of TB based on the Taiwan guidelines for TB diagnosis and treatment[21] and previous studies.[7,22] All chest X-rays were read in a double-blinded fashion by an infectious diseases specialist or a chest specialist, and by a radiologist. Briefly, five criteria for clinical diagnosis of TB were used: 1) patients with clinical symptoms and signs suggestive of TB diseases (such as sustained cough, weight loss, fever), chest X-rays that were typical of TB (typically showing a tuberculosis-like lesion), or a clinical response associated with administration of anti-tuberculous drugs, in the absence of other antimicrobial agents (clinical response was defined as improvement of chest X-ray/clinical symptoms); 2) clinical samples from

patients (including sputum, pleural fluid, gastric lavage fluid, BAL fluid or tissues) showing the presence of MTB in culture during the treatment period; 3) AFB smears or amplification of MTB complex by molecular methods of detection (PCR); 4) positive findings of TB on pathologic examination (caseating granulomas or AFB smear positive in the biopsy material); 5) samples positive by PCR, as well as AFB smear positivity.

Culture-confirmed tuberculosis

Culture-confirmed TB was based on a positive culture of MTB from any specimen that originated from a patient who had another positive culture during the six months of clinical suspicion of TB.

Statistical analyses

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of DTB were calculated in comparison with AFB smear microscopy or TB culture results and, subsequently, in comparison with AFB smear or clinical diagnosis. Statistical analyses were performed using the Statistical Package for the Social Sciences 15.0 statistics software (SPSS Inc – Chicago, IL, USA).

Results

Patients' disease status

This study analyzed data collected from 286 patients (120 patients who provided expectorated sputum samples and 166 patients who provided BAL samples). The final study sample comprised 198 males and 88 females, with a mean age of 64.01 ± 18.66 years (range: 1 to 97 years), and 159 patients (55.6%) were over 65 years old. All 286 patients enrolled in this study were tested by DTB, AFB smear microscopy, and culture. All patients were diagnosed with pulmonary disease based on abnormal chest X-ray findings, such as infiltrations, fibrosis, miliary lesions, pleural effusions, cavitations, calcifications, mass lesions, and nodular lesions. Of the 286 study patients with pulmonary disease, 62.6% (179/286) were positive by MTB culture and were considered to have a culture-confirmed diagnosis of TB, while 78.0% (223/286) of the patients fulfilled the criteria for a clinical diagnosis of TB (Table 1).

Performance of the DTB assay and AFB smear microscopy vs. culture in expectorated sputum samples

The expectorated sputum samples provided by 120 patients were tested by the DTB assay, AFB smear microscopy, and culture. Eighty six of these patients were positive by the MTB culture test and were considered to have a culture-confirmed TB, while 34 patients were negative by the MTB culture test (Table 2). A direct comparison of culture versus the use of AFB smear microscopy and the DTB assay in these patients was performed and showed that the DTB assay had a higher sensitivity than AFB smear microscopy (83.7% vs. 75.6%). The DTB assay also had a higher specificity than AFB smear microscopy (82.4% vs. 41.2%). The positive and negative predictive values (PPV and NPV) were higher for the DTB assay compared to the AFB smear microscopy (Table 2).

Performance of the DTB assay and AFB smear microscopy vs. clinical diagnosis in expectorated sputum samples

The clinical diagnosis was also used as the reference standard to compare the efficacy of AFB smear microscopy and the DTB assay in the 120 expectorated sputum samples (Table 2). The data showed that the DTB assay had a significantly higher sensitivity (77.2% vs. 70.3%) than AFB smear microscopy. The DTB assay also had a higher specificity (100% vs. 26.3%), and better NPV compared to AFB smear microscopy. Notably, the PPV of the DTB assay was 100% (Table 2).

Sensitivity of the AFB smear microscopy and DTB assay vs. culture in BAL specimens

The results of AFB smear microscopy and DTB assay were further compared in 166 BAL samples, with either the culture or the clinical diagnosis as a gold standard. Of the 166 patients who provided BAL samples, 93 patients were positive for MTB culture and were considered to have a culture-confirmed diagnosis of TB. The sensitivity and specificity of the DTB assay were demonstrated to be superior to those of the AFB smear microscopy. The positive and negative predictive values (PPV and NPV) were also higher for the DTB assay compared to AFB smear microscopy (Table 3).

Performance of the DTB assay and AFB smear microscopy vs. clinical diagnosis in BAL specimens

Clinical diagnosis was used as the gold standard to compare the efficacy of AFB smear microscopy and the DTB assay in the 166 BAL samples (Table 3). A total of 122 patients fulfilled the criteria for clinical diagnosis. The DTB assay had a significantly higher sensitivity (67.2% vs. 34.8%) than AFB smear microscopy. The DTB assay also had a higher specificity (100% vs. 26.3%), and better NPV compared to AFB smear microscopy (Table 3).

Table 1 - Patients' demographics

	n = 286
Age (years)	64.01 ± 18.66
Gender, n (%)	
Male	198 (69.2)
Female	88 (30.8)
Samples, n (%)	
Sputum specimens	120 (42.0)
BAL	166 (58.0)
Number of positive patients, n (%)	
Clinical diagnosis	223 (78.0)

BAL, bronchoalveolar lavage.

Table 2 - Detection of MTB in expectorated sputum samples (n = 120): comparison of AFB smear microscopy and DTB assay with culture or clinical diagnosis

	Culture		Sensitivity	Specificity	PPV	NPV
	Positive	Negative				
AFB						
Positive	65	20	75.6%	41.2%	76.5%	40.0%
Negative	21	14				
DTB						
Positive	72	6	83.7%.	82.4%	92.3%	66.7%
Negative	14	28				
	Clinical diagnosis		**Sensitivity**	**Specificity**	**PPV**	**NPV**
	Positive	**Negative**				
AFB						
Positive	71	14	70.3%	26.3%	83.5%	14.3%
Negative	30	5				
DTB						
Positive	78	0	77.2%	100%	100%	45.2%
Negative	23	19				

AFB, acid-fast bacilli; DTB, BDProbeTEC ET *Mycobacterium tuberculosis* complex direct detection assay; MTB, *M. tuberculosis*; PPV, positive predictive value; NPV, negative predictive value.

Table 3 - Detection of MTB in BAL specimens (n = 166): comparison of AFB smear microscopy and DTB assay with culture method and clinical diagnosis

	Culture		Sensitivity	Specificity	PPV	NPV
	Positive	Negative				
AFB						
Positive	39	9	41.9%	87.7%	81.3%	54.2%
Negative	54	64				
DTB						
Positive	77	5	82.8%	93.2%	93.9%	81.0%
Negative	16	68				
	Clinical diagnosis		**Sensitivity**	**Specificity**	**PPV**	**NPV**
	Positive	**Negative**				
AFB						
Positive	39	9	34.8%	79.5%	81.3%	29.7%
Negative	83	35				
DTB						
Positive	82	0	67.2%	100%	100%	52.4%
Negative	40	44				

AFB, acid-fast bacilli; DTB, BDProbeTEC ET *Mycobacterium tuberculosis* complex direct detection assay; BAL, bronchoalveolar lavage; MTB, *M. tuberculosis*; PPV, positive predictive value; NPV, negative predictive value.

Discussion

In this study, the effectiveness of the DTB assay in detecting MTB in expectorated sputum and in BAL specimens from patients with symptoms of a lung infection was evaluated. Since all the patients enrolled in this study had clinical symptoms and signs suggestive of TB, and patients were selected at the Division of Infectious Diseases, at the Division of Chest, the likelihood that the patients were truly infected with MTB was high. Not surprisingly, the percentage of respiratory specimens yielding positive TB culture results in the present study was 62.6%, compared to a recent multi-center trial, which found that 38.8% of the patients were smear and culture positive, while 11.9% of the patients were smear negative and culture positive.[23] The high yield of MTB on culture in the present study could also be due to the use of both liquid and solid media, as stipulated by the Centers for Disease Control of Taiwan, which reduces the time required for culture from six to two weeks, and also increases the positive rate of bacterial culture.

Since AFB smear microscopy had a sensitivity of only 75.6% compared to culture, and of 70.3% compared to clinical diagnosis in detecting MTB from the sputum samples, the use of only AFB smear microscopy for diagnosis would result in fewer than half of the TB patients being promptly isolated and treated. The DTB assay exhibited higher sensitivity and specificity than AFB smear microscopy when compared to culture as the reference standard. The DTB assay also had a higher sensitivity and specificity than AFB smear microscopy when compared to clinical diagnosis. The DTB assay sensitivity data fall within the range of previously reported values (82.7% to 97.6%).[12-14,16-18,24-31] Interestingly, six specimens were positive by the DTB assay but negative by culture. However, there were no DTB-positive specimens that were not also positive according to the criteria for clinical diagnosis, suggesting that the apparent false-positives seen on the DTB assay may actually represent false-negative culture results.

Treatment should be initiated to interrupt the transmission of MTB when the clinical presentation is highly suggestive of TB and the patient's result from DTB assay is positive (even the smear result is negative). However, if the patient's smear result is negative and clinical manifestations are uncertain, waiting until the culture results become available before beginning treatment is perhaps the better option.[32] TB transmission from patients with negative smear results is relatively low (approximately 17%).[33] A positive DTB result with a positive smear would indicate actual TB disease, and these patients should be isolated and receive antituberculous treatment until subsequent AFB smears are negative. However, a negative DTB result in a patient with an AFB-positive smear could indicate: 1) the presence of a NTM infection, and the patient could be released from isolation and avoid unnecessary anti-tuberculous treatment;[32,34] or 2) low specimen volume, low copy number of TB genes or due to the presence of unknown inhibitors in the sputum or BAL samples.

In this study, BAL samples were collected: 1) based on clinical symptoms suggestive of TB, 2) when sputum samples could not be obtained,[35-37] or 3) when a lung mass was observed (to differentiate TB from a malignancy).[34] PCR-restriction fragment length polymorphism has previously been used to detect mycobacterial species in bronchial washings, with a detection sensitivity > 80%.[22,38] However, only a few studies have reported the detection of MTB in BAL fluid by the DTB assay.[9,39] The present data suggest that the DTB assay offers an efficient alternative to AFB smear microscopy for the identification of MTB in BAL.

Although the cost of obtaining specimens by bronchoscopy and BAL is significantly higher than the costs associated with culture and identification (US$33 vs. US$13),[24] the superior performance and the shorter time required for the DTB assay (one to seven days for the DTB assay versus 28 to 50 days for culture and identification) are important advantages.

Among screening methods to detect pulmonary tuberculosis are AFB smear microscopy and culture on selective media in countries such as Nigeria,[40] and the QuantiFERON TB test in countries such as Trinidad and Tobago and France.[41,42] Although the QuantiFERON test is more expensive than the tuberculin skin test, the rapidity and sensitivity of the assay makes it very useful in a field setting. The GenXpert MTB/RIF assay, which was recently endorsed by the World Health Organization, rapidly detects the presence of MTB and can identify the mutations most frequently associated with rifampin resistance directly from smear-negative and smear-positive clinical sputum samples in less than two hours.[23,43] The GenXpert MTB/RIF assay system is more sensitive than the DTB system, although both methods require approximately the same time (14 days; sometimes 42 days when the bacterial culture is negative). However, although the DTB system is more economical, it is only applicable for the detection of pulmonary bacteria or for tissues with positive AFB smears, and cannot be used for extra-pulmonary tissues or tissues with negative AFB smear results.

It is important to note that, even though all study patients included in this study had a high clinical suspicion of TB at enrollment, the significant difference in NPV and PPV between the DTB assay and AFB smear microscopy validates the use of the DTB assay in these patients. Limitations of this study are: 1) not all sputum and BAL samples which were subjected to TB microscopy, TB culture and DTB were provided to the authors for this study, due to the standard operating procedure followed by the hospital; 2) the retrospective nature of the study. However, the strength of this study lies in the large number of expectorated sputum samples tested (120) and the number of BAL specimens tested (166), compared to previous studies.[36,35]

To summarize, it was demonstrated that early diagnosis using the DTB assay is a valuable tool for improving patient care and reducing transmission of TB, and for rapid diagnosis of MTB in the absence of sputum specimens. However, based on the present data, it is suggested that the DTB assay is insufficient as a single method for the diagnosis of TB; clinical diagnosis remains the ultimate guide in deciding the management of patients with TB.

Conflict of interest

All authors declare to have no conflict of interest.

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