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Brief Communication

## Performance of the BD ProbeTec ET direct detection assay for the analysis of *Mycobacterium tuberculosis* in respiratory and non-respiratory clinical specimens



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### Abstract

**Objectives:** Early detection of Mycobacterial tuberculosis infection (MTB) is pivotal for the treatment of tuberculosis (TB).

**Background:** This study was performed to evaluate the performance of BD ProbeTec ET direct detection assay (DTB) against the gold standard culture technique for confirmation of MTB infection.

**Methods:** A total of 266 consecutive and non-duplicate clinical specimens for detection of MTB were included in this study. There were 118 respiratory and 148 non-respiratory samples. All samples were tested by microscopy for acid-fast bacillus (AFB), MTB culture and biochemical identification with simultaneous testing by DTB.

**Results:** A total of 88 samples (33%) were culture-positive for MTB including 39/118 respiratory, 29/99 fluid and 20/49 tissue samples. DTB sensitivity for

respiratory samples was 97% and specificity was 96% with a positive predictive value (PPV) of 93% and negative predictive value (NPV) of 99%. Sensitivity of DTB in fluid samples was 80%, specificity 88%, PPV 69% and NPV 93% whereas sensitivity of DTB for tissue samples was 25%, specificity 90%, PPV 63% and NPV 63%. Of the 50 (56.8%) smear-positive samples, DTB sensitivity was 100% for respiratory, 85% for fluid and 100% for tissue samples.

**Conclusion:** DTB performed within acceptable limits for the rapid detection of MTB in respiratory samples compared to fluid and tissue specimens.

**Keywords:** Acid-fast bacillus; BD ProbeTec ET; Culture; DTB; *Mycobacterium tuberculosis*

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### Introduction

Infections with *Mycobacterium tuberculosis* (MTB) is conventionally diagnosed by smear and/or culture techniques. While the culture technique is still considered to be the gold standard, it yields results after weeks of delay and diagnosis by the smear technique lacks sensitivity that may

range from 50 to 80%.<sup>1</sup> Early detection of infection with MTB is of paramount importance for the immediate isolation and treatment of infected patients and their contacts. This may be possible by direct detection of tubercle bacilli in clinical samples by the application of different laboratory procedures such as amplification of mycobacterial DNA,<sup>2</sup> detection of specific lipids<sup>3</sup> and serological evidence for cell surface proteins.<sup>4</sup> Despite the availability of these methods, microscopy for acid-fast bacilli (AFB) remains the most cost effective and is a relatively simple method for direct detection of MTB in clinical specimens.<sup>5</sup>

Reliance on detection of MTB by microscopy is further complicated by the occurrence of mycobacteria species other than tubercle bacilli that may be responsible for 30% or more of mycobacterial infections.<sup>6</sup> Although these organisms are non-contagious, they may yield a positive acid-fast bacillus smear. It is therefore imperative that accurate diagnosis of MTB and *M. tuberculosis* complex (MTBC) be sought because of the infectious nature of the organism. It was for this reason that a number of techniques based on polymerase chain reaction (PCR) amplification of *Mycobacterium* specific nucleic acids were introduced<sup>7–10</sup> for the direct detection of MTB; this includes the BD ProbeTec ET MBTC direct detection assay (DTB).<sup>11</sup> The DTB assay is a semi-automated real-time molecular technique that targets MTB specific DNA (IS6110) using specific primers and fluorescently tagged probes. Evaluation of the diagnostic efficacy of the DTB assay in pulmonary samples such as bronchoalveolar lavage and sputum has clearly documented better performance of the DTB assay compared to smear microscopy.<sup>12</sup> This study was performed to assess the performance of DTB with regards to detection of MTBC in respiratory and non-respiratory clinical samples from patients suffering from tuberculosis at King Khalid University Hospital.

## Materials and Methods

This was a prospective study of 266 consecutive and non-duplicate clinical specimens received in the laboratory at King Khalid University Hospital between June 2009 and December 2011 for detection of MTB. Among these samples, there were 118 respiratory and 148 non-respiratory specimens, comprising 99 samples from sterile body fluid sites and 49 samples from various tissues. All samples from non-sterile body sites were decontaminated with *N*-acetyl-L-cysteine–NaOH (2% final concentration) and concentrated by centrifugation by the same technical staff. Samples from sterile sites were homogenized when required prior to re-suspension in normal saline and concentrated by centrifugation. All of the specimens were processed in accordance with the laboratory policy for AFB smear microscopy and MTB culture using both solid (Lowenstein-Jensen) medium and liquid Mycobacteria Growth Indicator Tube (MGIT; BD Biosciences, Sparks MD) medium. Identification of MTBC was finally performed by biochemical testing. All the samples were simultaneously tested by DTB. After the completion of all procedures, results from microscopy, culture and DTB were collated and evaluated.

## BD ProbeTec ET direct TB (DTB) assay

Initial processing of the samples was performed in a class 2 biological safety cabinet within a category 3 containment laboratory. The BD ProbeTec ET Direct TB Assay was performed in accordance with the manufacturers' instructions as described previously.<sup>13</sup> Briefly, a 500 µl aliquot of decontaminated sample was added to 1 ml of wash buffer and the contents were mixed by vortexing, which was followed by centrifugation in a microfuge. Cells in the pellet were rendered non-viable by heating in a self-contained oven. Following that, the pellet was resuspended in 100 µl of lysis buffer. The contents were mixed and placed in a sonic water bath for 45 min, followed by another round of centrifugation. The pellet was resuspended again in 600 µl of sample neutralization buffer and the mixture vortexed, centrifuged and analysed. Positive and negative controls supplied by the manufacturers were also prepared simultaneously. An eight channel ProbeTec pipette was used to dispense 150 µl of each sample into a microwell of the priming plate and incubated for 20 min at room temperature. After the incubation, the priming plate was placed on a heating block at 53.5 °C for 10 min for pre-warming, followed by the transfer of 100 µl of sample from each priming microwell to an amplification microwell. The wells were sealed and placed into the BD ProbeTec ET instrument for 60 min. After one hour, Metric Other Than Acceleration (MOTA) values were printed by the instrument for each sample and control. MOTA values higher than 3400 were considered positive for MTBC DNA. The result was considered negative if the MTB MOTA value was less than 3400 and the Internal Amplification Control (IAC) was more than 5000. The results with MTB MOTA values of less than 3400, and IAC less than 5000 were interpreted as sample inhibition. The duration of the DTB assay for confirmation of TB diagnosis was between three to four hours that was markedly shorter than the culture method that usually takes six to eight weeks.

## Results

Of the 266 total clinical samples tested, 88 (33%) yielded evidence of the presence of MTB by the culture technique. Table 1 describes data for the ability of the DTB assay to detect MTBC among samples from respiratory and non-respiratory sites, which tested either positive or negative by the culture technique which is considered the gold standard. Of the total of 118 respiratory samples, MBT was found in 39 samples by culture, whereas the remaining 79 samples yielded no evidence of MTB. DTB detected MTBC in 38 out of 39 culture-positive specimens whereas 3 of 79 culture-negative samples were tested positive by DTB. Of the 99 fluid samples from sterile body sites 29 samples were culture-positive whereas 70 samples tested negative by the culture technique. Of the 29 culture-positive fluid samples, the DTB was able to detect MTBC in only 20 samples, whereas in 70 culture-negative samples, 5 samples yielded a positive result when tested by the DTB. For the tissue samples, of the 49 total samples, only 20 grew MTB, and of these 20 samples, the DTB was able to detect MTBC in only 5 samples. Of the remaining 29 samples tested negatively by culture, the DTB could detect MTBC in 3 samples.

**Table 1: *Mycobacterium tuberculosis* detection by DTB and culture technique.**

Specimen type	Culture	Culture		Total	
		Positive	Negative		
Respiratory	DTB	Negative	1	76	77
		Positive	38	3	41
		<b>Total</b>	<b>39</b>	<b>79</b>	<b>118</b>
Fluid	DTB	Negative	9	65	74
		Positive	20	5	25
		<b>Total</b>	<b>29</b>	<b>70</b>	<b>99</b>
Tissue	DTB	Negative	15	26	41
		Positive	5	3	8
		<b>Total</b>	<b>20</b>	<b>29</b>	<b>49</b>
All samples	DTB	Negative	25	167	192
		Positive	63	11	74
		<b>Total</b>	<b>88</b>	<b>178</b>	<b>266</b>

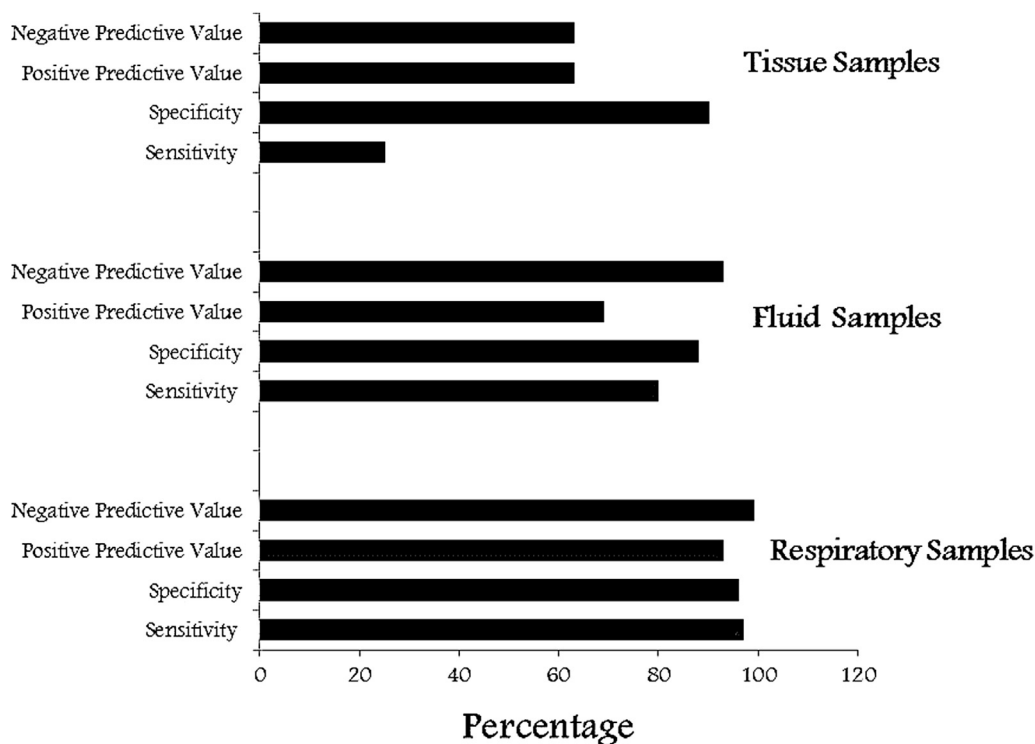
Figure 1 shows data for DTB performance against the gold standard culture technique for detection of MTB. Among the three types of samples tested, the efficiency of DTB in detection of MTBC was remarkably high in respiratory samples where its sensitivity was 97% and specificity was 96%, with a positive predictive value of 93% and a negative predictive value of 99%. DTB performance in detection of MTBC in fluid samples was found to be better than its ability to detect MTBC in tissue samples. This was evident by a sensitivity of 80% and specificity of 88%, with a positive predictive value of 69% and a negative predictive value of 93% in fluid samples

compared to the tissue samples where its sensitivity was 25% and specificity was 90%, with a positive predictive value of 63% and negative predictive value of 63%.

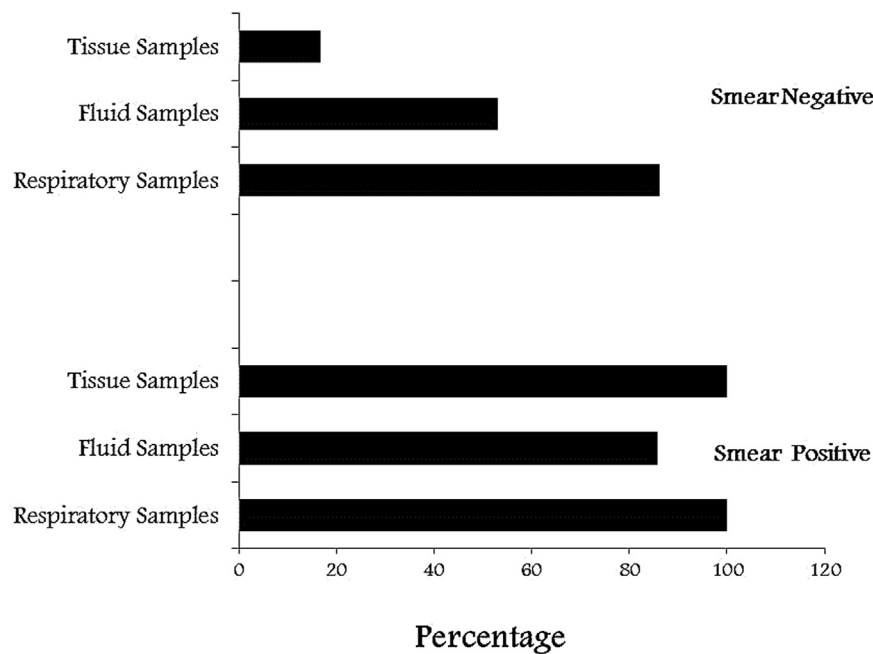
Of the 88 total culture-positive clinical samples, AFB in smear microscopy was detected in 50 (56.8%). Among 118 respiratory samples, 33 (28%) were AFB smear-positive, and of the 99 fluid samples 15 (15%) were smear-positive, whereas in 49 tissue samples only 2 (4%) samples yielded direct evidence of the presence of AFB by microscopy. Figure 2 describes data for DTB sensitivity among smear-positive and smear-negative samples in all categories. Sensitivity of the DTB among the smear-positive respiratory samples was 100%, whereas in smear-negative respiratory samples, the sample had a sensitivity of 86%. Among the fluid samples, the sensitivities of smear-positive and smear-negative samples were 85.7% and 53%, respectively, whereas in smear-positive and smear-negative tissue samples, these were 100% and 16.6%.

## Discussion

Performance of DTB for rapid detection of MTBC against the gold-standard culture technique in clinical respiratory samples was better in comparison to non-respiratory samples. To provide rapid and accurate results it is necessary for a diagnostic test should have high sensitivity and specificity. The sensitivity (97%), specificity (96%), positive predictive value (93%) and negative predictive value (99%) of DTB in the detection of MTBC in the present study of respiratory samples was better than the previously



**Figure 1:** Performance of the BD ProbeTec ET Direct TB (DTB) assay against the gold standard culture technique for the detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory samples.



**Figure 2:** Sensitivity of the BD ProbeTec ET Direct TB (DTB) assay among acid-fast bacillus smear-positive and smear-negative sample in all categories.

reported sensitivity, specificity, positive predictive value and negative predictive values of 89.7%, 93.7%, 85.4%, and 95.7%, respectively. Sensitivity of DTB as low as 63.2% has also been reported<sup>14</sup> which appears to be lower than a number of other studies reporting sensitivity of the DTB ranging from 82.7% to 100% for the detection of MTBC in respiratory specimens.<sup>15,16</sup> The findings of this study are consistent with previous reports of DTB being a highly sensitive and specific test with a higher negative predictive value of 99.5%,<sup>17</sup> making it a powerful tool for diagnosis or exclusion of tuberculosis in respiratory specimens.

Despite its excellent performance in respiratory samples, the performance of the DTB in extra-pulmonary specimens, particularly in tissue samples from extra-pulmonary sites was not comparable. The sensitivity and specificity of DTB in detecting MTBC in tissue samples in this study was 25% and 90%, respectively. Similar results were also observed by application of DTB in a study examining formalin fixed paraffin embedded tissue samples with necrotizing granulomatous inflammation, where the sensitivity and specificity of DTB was 40% and 100%, respectively.<sup>18</sup> The low sensitivity in tissue samples may possibly be due to several purification and preparation steps involved in the preparation of the tissue samples before evaluation. Moreover, clinical samples, particularly respiratory samples, are known to harbour interfering substances responsible for false negative results in detection of mycobacterial DNA<sup>19</sup> that may contribute to decreased sensitivity of PCR based detection of MTB. On the contrary, the sensitivity of DTB in smear-positive respiratory samples including tissue samples was 100% in this study where the DTB detected MTBC in 28 of 33 samples. In agreement with findings from the present study, of 20 tissue specimens from patients with a confirmed diagnosis of tuberculosis, the DTB has been shown to detect MTBC in 18 clinical samples with 100%

specificity.<sup>18</sup> Similarly, DTB has been reported to exhibit an overall sensitivity, specificity, positive predictive value and negative predictive value of 85%, 100%, 100% and 99%, respectively for the detection of MTB in both respiratory and non-respiratory samples.<sup>20</sup> Despite the technical hazards and low sensitivity in tissue samples observed in the present study, DTB appears to be a highly sensitive and specific assay that is capable of detecting MTB accurately in clinical samples within three to four hours. The low sensitivity of DTB in tissue samples could possibly be due to a lack of standardization of the extraction procedure for DNA from tissues.

Of the total of 178 culture-negative clinical specimens, 11 (6.1%) samples yielded positive results by DTB in this study. DTB related false-positive results reported in the past were frequently obtained in patients either receiving treatment for pulmonary tuberculosis, old pulmonary tuberculosis patients or due to the presence of *M. abscessus* in the clinical samples.<sup>21</sup> It is possible that the detection of dead mycobacteria in specimens detected by DTB may have yielded false positive results. Since the present study was conducted utilizing clinical specimens received in the microbiology laboratory and lacked clinical details, it was difficult to ascertain the current status of therapeutic intervention. In addition, lower positive predictive value of DTB particularly among non-respiratory specimens could be due to a higher number of false positive results. Similarly, 25 of the 88 total culture-positive specimens in the present study yielded false negative results particularly among the tissue samples where DTB failed to detect MTBC in 15 out of 20 culture-positive samples. In addition to the presence of interfering substances in the clinical samples,<sup>19</sup> the most likely factors contributing to false negative results may include the presence of small numbers of mycobacteria, suboptimal target extraction or unequal distribution of test material.

In conclusion DTB assay performance was comparable with the gold standard culture method for early detection of MTB in clinical samples. This study was however limited by relatively small numbers conducted at a single centre. Large-scale studies are recommended in the Kingdom to validate the findings of the present study. Moreover, being that a molecular assay uses DTB, this may prove to not be a cost effective approach in early case detection in a community setting.

#### Conflict of interest

The authors have no conflict of interest to declare.

#### Authors' contributions

All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript. ASM, Study design and laboratory investigation, data collection and analysis, literature review, manuscript preparation. HHA, Literature review, manuscript preparation. MSS, Collected and organized data, literature review, manuscript preparation. NAZ, Manuscript preparation. RAM, Manuscript preparation. ZSA, Study design, literature review, manuscript preparation and provided logistic support.

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