

Evaluation of the BDProbeTec™ ET System for the Direct Detection of *Mycobacterium tuberculosis* Complex from Clinical Respiratory Specimens using Strand Displacement Amplification Technology

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ABSTRACT

BACKGROUND: The increase of tuberculosis (TB) has been compounded by the emergence of multi-drug resistant *M. tuberculosis* strains which have contributed to an increased case-fatality rate of TB. This situation presents a need for more rapid diagnostic methods in order to diagnose and treat more quickly. New technologies involving DNA amplification techniques have provided an opportunity for more rapid diagnosis.

METHODS: This evaluation has examined the performance of homogenous Strand Displacement Amplification (SDA) and fluorescent energy transfer detection on an instrumented system. An internal amplification control is run with each sample and is designed to verify the SDA reaction. To date, 727 well-characterized respiratory specimens sent to our laboratory for routine testing have been analyzed. A total of 674 specimens were from patients not treated for TB, or treated for less than 7 days. After the standard NALC-NaOH decontamination procedure, liquid (MGIT 960) and solid (Löwenstein Jensen, Middlebrook) media were inoculated. Sensitivity and specificity of the direct TB (DTB) assay were estimated, when compared to the conventional culture media.

RESULTS: Initial overall sensitivity and specificity of the BDProbeTec™ ET System were 91.5% and 94.4%, respectively. Of the 82 culture-positive specimens 75 were DTB positive, and of the 645 culture negative specimens 609 were DTB negative. For smear positive specimens the sensitivity and specificity of the DTB assay were 100% each. For smear negative specimens the sensitivity and specificity were 85.1% and 94.4%, respectively. However, false positive DTB results were later proven to be true positive by follow up cultures, resulting in a final sensitivity of 87.9% and a specificity of 96.0% for smear negative specimens. The internal amplification controls indicated that no inhibition of the SDA reaction was detected in any specimen.

CONCLUSIONS: The BDProbeTec™ ET System is easy to use with good sensitivity and specificity for the rapid direct detection of *M. tuberculosis* complex, even in smear negative specimens.



INTRODUCTION

■ Historically, *M. tuberculosis* has been diagnosed by conventional culture media such as egg-based Löwenstein-Jensen and Middlebrook agar medium. Recovery of mycobacteria with these methods typically takes 2–4 weeks. Broth based media such as BACTEC™ MGIT™ 960 System have decreased the recovery time to 7–14 days.

■ The resurgence of tuberculosis in recent years has been compounded by the emergence of multi-drug resistant strains, which have contributed to an increase of case-fatality rate of TB. This situation presents a need for rapid diagnostic in order to diagnose and treat patients more quickly. New technologies involving DNA amplification techniques have provided an opportunity for more rapid detection of *M. tuberculosis*.

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MATERIALS AND METHODS

■ This evaluation has examined the performance of Strand Displacement Amplification (SDA) which is an isothermal DNA amplification technology on an instrumented system. This system, known as BDProbeTec™ ET System, is based on simultaneous amplification and detection of target DNA by the use of amplification primers along with a fluorescent-labeled detector probe. An internal amplification control is run with each sample and is designed to detect samples that may inhibit the SDA reaction. This reduces the possibility of reporting a false negative result due to inhibitors and confirms the validity of the amplification reaction. The system can perform up to 96 tests simultaneously.

■ A total of 727 well characterized respiratory specimens sent to our laboratory for routine testing have been analyzed. After the standard NALC-NaOH decontamination procedure, liquid (MGIT 960) and solid (Löwenstein-Jensen, Middlebrook) media were inoculated. Sensitivity and specificity values of the direct TB (DTB) assay were estimated, when compared to the conventional culture media and to the resolved results for the clinical diagnosis of tuberculosis.

RESULTS

Table 1. Comparison of SDA results with culture results for detection of *M. tuberculosis* complex

Group of specimens SDA results	No. of specimens with culture results		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Total (n=727)			91.5	94.4
Positive	75	36		
Negative	7	609		
Smear positive (n=37)			100	100
Positive	35	0		
Negative	0	2*		
Smear negative (n=690)			85.1	94.4
Positive	40	36		
Negative	7	607		

**M. kansasii*, *M. szulgai*

Table 2. Comparison of SDA results with culture and resolved results for the clinical diagnosis of tuberculosis

Group of specimens SDA results	No. of specimens with culture and resolved results		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Total (n=727)			92.5	96.1
Positive	86	25		
Negative	7	609		
Smear positive (n=37)			100	100
Positive	35	0		
Negative	0	2*		
Smear negative (n=690)			87.9	96.0
Positive	51	25		
Negative	7	607		

**M. kansasii*, *M. szulgai*

Results including specimens from patients under therapy more than 7 days

■ Initial overall sensitivity and specificity of the BDProbeTec™ ET System for the 727 specimens analyzed were 91.5 and 94.4%, respectively. Of the 82 culture positive specimens 75 were DTB positive, and of the 645 culture negative specimens 609 were DTB negative. For smear positive specimens the sensitivity and specificity of the DTB assay were 100% each, and for smear negative specimens 85.1 and 94.4%, respectively. The 2 specimens yielding isolates of NTM, *M. kansasii* and *M. szulgai*, were correct negative by the DTB assay (Table 1).

■ After combining culture results and clinical data for the patients the sensitivity and specificity for the DTB test were 92.5 and 96.1%, respectively (Table 2). A total of 86 specimens were collected from patients with a diagnosis of pulmonary tuberculosis, in accordance with a positive rate of 11.8%.

■ The internal amplification controls indicated that no inhibition of the SDA reaction was detected in any specimen.

Table 3. Comparison of SDA results with culture results for detection of *M. tuberculosis* complex excluding specimens from patients under therapy > 7 days

Group of specimens SDA results	No. of specimens with culture results		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Total (n=674)			91.8	95.6
Positive	56	27		
Negative	5	586		
Smear positive (n=29)			100	100
Positive	28	0		
Negative	0	1*		
Smear negative (n=645)			84.8	95.6
Positive	28	27		
Negative	5	585		

**M. kansasii*

Table 4. Comparison of SDA results with culture and resolved results for the clinical diagnosis of tuberculosis excluding specimens from patients under therapy >7 days

Group of specimens SDA results	No. of specimens with culture and resolved results		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Total (n=674)			92.4	96.4
Positive	61	22		
Negative	5	586		
Smear positive (n=29)			100	100
Positive	28	0		
Negative	0	1*		
Smear negative (n=645)			86.8	96.4
Positive	33	22		
Negative	5	585		

**M. kansasii*

Results excluding specimens from patients under therapy more than 7 days

■ Out of the 727 specimens 674 were collected from patients receiving no or not more than 7 days treatment for tuberculosis. Of the 61 culture positive results 56 were DTB positive and out of the 613 culture negative specimens 586 were DTB negative. Compared to the culture results the sensitivity and specificity was 91.8 and 95.6%, respectively (Table 3).

■ A total of 61 specimens were from patients with a diagnosis of tuberculosis on the basis of clinical and microbiological findings. Of the 28 samples which were smear and culture positive for *M. tuberculosis*, all were DTB positive. Thirty-eight specimens were smear negative but culture positive; 33 were DTB positive. The sensitivity and specificity were 92.4 and 96.4%, respectively for the resolved data (Table 4).

CONCLUSIONS

■ The BDProbeTec™ ET System is a sensitive and specific method for the rapid direct detection of *M. tuberculosis*, even in smear negative specimens. Although at present amplification assays cannot replace the conventional culture methods, the SDA assay was found to be highly sensitive and specific for the detection of *M. tuberculosis* in pulmonary specimens. The assay protocol was easy to perform and was suitable for a routine bacteriology laboratory's work flow.

