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An evaluation of the BD ProbeTec ET system for the direct detection of *Mycobacterium tuberculosis* in respiratory samples

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In controlling the spread of tuberculosis, early detection of disease caused by organisms of the *Mycobacterium tuberculosis* complex (MTBC) is vital. The BD ProbeTec ET system provides a method for the direct detection of MTBC by strand displacement amplification. Two hundred and five respiratory samples from patients with a high probability of tuberculosis were assessed by ProbeTec and by microscopy and culture for mycobacteria. ProbeTec positive results were obtained with 101 of 109 samples from which MTBC organisms were isolated. ProbeTec correctly signalled 78 of 81 samples that gave growths of mycobacteria other than tubercle bacilli (MOTT) as negative. Three samples gave false-positive results, corrected on repeat testing. Positive and negative predictive values (PPV, NPV) were 0.97 and 0.90 and the system showed a sensitivity and specificity of 92.7% and 96.0%, respectively. These values rose to PPV 0.97, NPV 0.96, sensitivity 97.1% and specificity 96.0% when data from the small number of gastric lavage samples tested were removed from the analysis. The BD ProbeTec ET system offers a robust and reliable molecular biological approach to the detection of MTBC organisms in respiratory samples in a semi-automated format.

Introduction

The global prevalence of mycobacterial infection has been estimated to be 32% (1.9 billion people) with 8 million new cases of tuberculosis diagnosed annually and an average case fatality rate of 23% [1]. Although disease rates may be generally lower in the developed world, many countries, including the UK, suffer a high incidence of tuberculosis in certain population groups [2]. The control and eventual elimination of tuberculosis requires that a high priority be placed on case finding, contact tracing and prompt initiation of treatment.

Early detection of pulmonary disease aids the timely initiation of treatment and triggers prompt activation of contact tracing procedures, interventions that can halt the chain of transmission. Thus, direct detection of tubercle bacilli in respiratory samples is of great importance. This is possible with various laboratory methods including molecular amplification of mycobacterial DNA [3], recognition of characteristic lipids [4] and, potentially, serology for cell surface protein fractions [5]. However, microscopy for acid-fast bacilli (AFB) remains the simplest and most cost-effective method [6]. The sensitivity of microscopy relative to culture may vary from 50% in some studies to >80% in others [7]. Furthermore, while it is held that patients with sputum smear-negative tuberculosis are less infectious, empirical evidence suggests that they can still transmit *Mycobacterium tuberculosis* [8]. A further complication, particularly in developed countries, is the occurrence of mycobacteria other than tubercle bacilli (MOTT) species in respiratory samples, accounting for 30% or more of infections [9]. Although not contagious, these organisms may give rise to a positive AFB smear. Despite anecdotal claims to the contrary, the reliable separation of *M. tuberculosis* complex (MTBC) strains from MOTT species on the basis of microscopic appearance alone is potentially misleading and smear positivity may not indicate MTBC and, therefore, infectiousness. In contrast, contact tracing may give rise to undue concern if initiated on smear positivity subsequently found to be due to a MOTT strain. Thus, acid-fast microscopy may be of high sensitivity but only moderate specificity.

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Therefore, as an adjunct to the acid-fast smear, it would be valuable to apply a procedure that is highly specific for MTBC organisms. In this context, a PCR-based amplification technique is usually applied. However, PCR is prone to a number of difficulties both technical (e.g., the inhibitory effects of clinical samples) and organisational (the need for dedicated facilities and personnel) which contribute to a high test cost [10, 11]. Strand displacement amplification (SDA [12]) offers sensitivity that is comparable with if not greater than PCR-based techniques. The BD ProbeTec ET^{TM} system (Becton Dickinson, Oxford) uses SDA in a partially automated format, lessening the requirement for specialist personnel and dedicated facilities [13]. This study evaluated the ProbeTec ET system in a busy diagnostic mycobacteriology laboratory.

Materials and methods

Samples

To maximise positive findings, respiratory samples from patients with a high index of clinical suspicion of tuberculosis were selected for study based upon previously described criteria [14]. Data on samples from patients known, or later discovered, to be established on anti-tuberculous therapy were removed from the analysis. All samples were liquefied with dithiothreitol and concentrated by centrifugation (3000 *g*) before examination by fluorescence microscopy for acid-fast organisms (Auramine-phenol staining). Samples were then decontaminated with NaOH 4% w/v with intermittent agitation and again concentrated before inoculation of both solid (Löwenstein Jensen) medium and liquid culture medium (BacT/ALERT 3D, bioMérieux UK, Basingstoke) for the isolation of mycobacteria. At this point also a portion of the prepared sample was set aside for processing by the ProbeTec system. Isolates of mycobacteria were identified by standard phenotypic methods and by the application of DNA probes (Accuprobe; GenProbe, San Diego, CA, USA).

BD ProbeTec method

All initial stages of this process were performed in a class 1 biological safety cabinet within a category 3 containment laboratory. The BD ProbeTec ET Direct TB Assay was performed according to the manufacturer's directions. Briefly, a 500-μl portion of the digested, decontaminated, concentrated sample as prepared for culture was added to 1 ml of wash buffer and vortex mixed before centrifugation at 12 200 *g* for 3 min in a microfuge with an aerosol-protected rotor. Cells present in the deposit were disrupted to release DNA by heating in an oven at 105°C for 50 min followed by re-suspension in 100 μl of lysis buffer, mixing and treatment in a sonic bath for 45 min at 65°C. Further processing did not require the use of a biological safety cabinet. After a further pulse centrifugation, 600 μl of neutralisation buffer were added to each tube. Positive and negative controls as supplied with each reagent batch were also prepared. One portion of the prepared sample was set aside for storage at −20°C. The programmable eight-channel ProbeTec pipettor with aerosol-resistant tips was used to dispense 150 μl of each sample into a microwell of the priming plate. After incubation at room temperature for 20 min, the priming plate was transferred to a heating block, set at 72.5°C, for 10 min after which 100 μl of sample were transferred to a microwell of a pre-warmed amplification plate. When all samples had been transferred the amplification plate was immediately placed in the ProbeTec Amplification/Reader instrument for 60 min. Amplification and detection take place simultaneously and results are automatically printed when this period is complete.

Each test well contains an internal control and separate positive and negative control tubes were included with each test run. Samples that gave MOTA (metric other than acceleration) readings >3400 were recorded as positive for MTBC regardless of the internal amplification control (IAC) MOTA. If the MTBC MOTA was <3400 and that of the IAC MOTA was >5000, the sample was considered negative for MTBC. If the MTBC MOTA was <3400 and the IAC MOTA was <5000, the result was indeterminate and the sample was re-tested. If, on re-testing, the result remained indeterminate, it was considered that inhibitors in the sample had suppressed amplification. After freezing and thawing in an attempt to reduce inhibition the sample was again re-tested.

When all procedures were complete, results of microscopy, culture and SDA were collated and evaluated.

Results

In total, 205 respiratory samples from patients selected as having a high probability of mycobacterial infection were tested by all three methods (smear, culture and ProbeTec ET). Of these, 190 samples were found to be positive for mycobacteria on culture. MTBC organisms were isolated from 109 samples, MOTT organisms from 81 and 15 were negative (Table 1).

Table 1. Summary of culture results

Parameter	Number of samples
Total samples tested	205
Culture-negative for mycobacteria	15
Culture-positive for MTBC	109
Culture-positive for MOTT	81
M. avium complex	42
M. malmoense	24
M. kansasii	6
M. gordonae	3
M. chelonae	5
M. xenopi	1

Analysis of the 109 samples that yielded growths of *M. tuberculosis* complex organisms (Table 2) showed that 100 were correctly identified as positive in the ProbeTec system, including 99 of the 101 samples in which AFB were detected. One sample initially showed inhibition but gave a positive reaction on repeat testing. Eight samples gave anomalous results, in that they were culture positive for MTBC organisms but negative in the ProbeTec system. Of these samples, one was a smear-positive broncho-alveolar lavage (BAL) which remained negative on repeat testing and five were gastric lavage samples from the same child.

Of 81 samples found to give growths of MOTT organisms, 55 were found to be negative in the ProbeTec system and 23 showed inhibition. Six of the 23 inhibited samples gave negative test results on repeat testing. However, there were three false-positive ProbeTec results, all from samples that subsequently grew MOTT species. All three samples gave negative results on repeat testing (Table 3). None of the 15 culture-negative samples gave positive reactions with ProbeTec, although four showed irreversible inhibition (Table 4). Four samples were smear-positive but culture-negative despite prolonged incubation in both liquid and solid culture systems. All four were BAL samples and this finding probably represents the presence of non-viable environmental mycobacteria.

Overall results are summarised in Table 5. These data give positive and negative predictive values (PPV, NPV) of 0.97 and 0.90, respectively with a test sensitivity of 92.7% and a specificity of 96.0%. If the six gastric lavage samples are removed from the assessment, these figures become PPV 0.97, NPV 0.96, sensitivity 97.1% and specificity 96.0%, respectively (Table 6). Furthermore, if the technical difficulty causing two of the three false-positive results is condoned, the specificity rises to 98.6%.

Table 2. ProbeTec ET and AFB smear results with samples culture-positive for MTBC

AFB smear result	ProbeTec ET result			
	Positive	Negative	Inhibited	Total
Negative	1*	7†	0	8
Positive	99	1‡	1§	101
Total	100	8	1	109

*Gastric washing from a 10-year-old child.
†Includes five gastric washings from the same child.
‡BAL sample remained negative on repeat testing.
§Multi-drug-resistant TB case, positive result on repeat testing.

Table 3. ProbeTec ET and AFB smear results with samples culture-positive for MOTT organisms

AFB smear result	ProbeTec ET result			
	Positive	Negative	Inhibited	Total
Negative	1*	16	4	21
Positive	2†	39	19	60
Total	3	55	23‡	81

*The system called this positive but with a very low score, negative on repeat (*M. gordonae*).
†Both tests negative on repeat (*M. avium* complex, *M. malmoense*).
‡Six of 23 tests negative on repeat.

Table 4. ProbeTec ET and AFB smear results with samples culture-negative for mycobacteria

AFB smear result	ProbeTec ET result			
	Positive	Negative	Inhibited	Total
Negative	0	7	2	9
Positive	0	4*	2	6
Total	0	11	4†	15

*BAL samples, culture-negative after prolonged incubation, probably non-viable environmental organisms.
†Still inhibitory on repeat.

Table 5. Corrected (validated) results on 205 samples

Parameter	Number of samples
Valid ProbeTec positive	101
Valid ProbeTec negative	72
Anomalous positive finding	3
Anomalous negative finding	8
Irreversible inhibition present	
MTBC on culture	0
culture-negative for MTBC	21

Discussion

In controlling the transmission of tuberculosis within communities, the detection of mycobacterial infection by acid-fast smear remains crucial. However, the presence of AFB on microscopy may be due to many different mycobacterial species, especially in developed countries with a low incidence of tuberculosis. Confirmation that infection is due to organisms of the MTBC, thus stimulating the initiation of control measures, is an important purpose underlying the application of molecular biological techniques to respiratory samples [15]. In other studies of the application of SDA to the direct detection of MTBC organisms in clinical samples, low numbers of positive specimens have been available. Down *et al.* [16] did not assess the system on samples that yielded isolates of MOTT organisms and Bergmann *et al.* [17], despite testing 600 samples, found only 57 that gave growths of mycobacteria of which only 16 were MTBC organisms. In the present study the selection criteria

Table 6. ProbeTec results validated against culture outcome

Parameter	All samples	Excluding gastric washings
PPV	0.97	0.97
NPV	0.90	0.96
Sensitivity	92.7%	97.1%
Specificity	96.0%	96.0%

resulted in an analysis which was based on a large number of samples that gave growths of mycobacteria.

Only one of 101 smear- and culture-positive MTBC samples gave negative results by ProbeTec. A further seven samples were culture-positive but smear-negative for MTBC organisms; however, five of these were gastric lavage samples. Gastric lavage may be the only useful strategy in attempting the laboratory diagnosis of respiratory tuberculosis in young children but this procedure is unlikely to be helpful in older children and adults [18]. Although it is interesting that one gastric washing sample resulted in a positive test by ProbeTec, we consider that such specimens are, in general, inappropriate for molecular tests.

Inhibitory samples are frequently a problem in the application of molecular techniques [3]. Re-testing of such samples after freezing and thawing is often successful in removing inhibition and 7 of the 28 inhibitory tests in this study were resolved in this way (including the only positive sample to show inhibition). Thus, none of the MTBC-positive samples remained undetected because of inhibition. It is interesting that of the 21 samples showing irreversible inhibition, all were sputum samples and 17 gave growths of MOTT organisms, amounting to 21% of all samples from which these organisms were isolated. The possibility that the presence of these bacilli causes an alteration of sample composition resulting in a greater likelihood of inhibition in molecular tests merits further study. Previous studies have either eliminated inhibitory samples from their analysis [17] or not examined samples that gave growths of MOTT organisms [16].

Three samples gave positive results, although subsequently found to contain MOTT organisms. These false-positive findings were reversed on repeat testing but the results of the initial test may have led to confusion and positive findings may not be questioned in routine testing. We believe that two of these false-positive results were due to a technical error resulting in the carry-over of material from an adjacent well of the microtitration plate. A modification of the handling protocol successfully eliminated this potential problem in all subsequent tests. This involved altering the direction of travel of the multi-pipettor and the use of an empty row of wells between each test row, thus avoiding the possibility of sample cross-over.

This study demonstrates that ProbeTec ET offers a high level of sensitivity and specificity with excellent PPV and NPV for the direct detection of MTBC organisms in respiratory samples as compared with the ‘gold standard’ of culture. PCR-based procedures, in addition to frequent problems of sample inhibition, require a separate suite of rooms and specifically assigned staff. In contrast, because of the automation of several of the reaction stages, ProbeTec ET reduces this requirement for dedicated facilities and specialist personnel. These features, in addition to the use of a ‘tear-off’ microtitration tray format, mean that ProbeTec is likely to offer a lower cost per test than PCR methods in routine laboratory applications. Therefore, we believe that BD ProbeTec ET has the potential to overcome many of the problems associated with the application of PCR in diagnostic laboratories and represents a significant development in the diagnosis and control of tuberculosis.

References

1. World Health Organization. Global tuberculosis control. World Health Organization, 2000; document no. WHO/CDS/TB/2000.275.
2. Rose AMC, Watson JM, Graham C *et al.* Tuberculosis at the end of the 20th century in England and Wales: results of a national survey in 1998. *Thorax* 2001; **56**: 173–179.
3. Kearns AM, Freeman R, Steward M, Magee JG. A rapid polymerase chain reaction technique for detecting *M. tuberculosis* in a variety of clinical specimens. *J Clin Pathol* 1998; **51**: 922–924.
4. French GL, Chan CY, Cheung SW, Oo KT. Diagnosis of pulmonary tuberculosis by detection of tuberculostearic acid in sputum by using gas chromatography-mass spectrometry with selected ion monitoring. *J Infect Dis* 1987; **156**: 356–362.
5. Gennaro ML. Immunologic diagnosis of tuberculosis. *Clin Infect Dis* 2000; **30** Suppl 3: S243–S246.
6. Salfinger M. Diagnosis of tuberculosis and other diseases caused by mycobacteria. *Infection* 1997; **25**: 60–62.
7. Freeman R, Magee JG, Watt B, Rayner AB. Being positive about the smear. *Thorax* 2001; **56**: 417–418.
8. Behr MA, Warren SA, Salamon H *et al.* Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet* 1999; **353**: 444–449.
9. Lamden K, Watson JM, Knerer G, Ryan MJ, Jenkins PA. Opportunist mycobacteria in England and Wales: 1982–1994. *Commun Dis Rep CDR Review* 1996; **11**: R147–R151.
10. Noordhoek GT, Kolk AHJ, Bjune G *et al.* Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. *J Clin Microbiol* 1994; **32**: 277–284.
11. Hawkey PM. The role of polymerase chain reaction in the diagnosis of mycobacterial infections. *Rev Med Microbiol* 1994; **5**: 21–32.
12. Walker GT, Fraiser MS, Schram JL *et al.* Strand displacement amplification – an isothermal, *in vitro* DNA amplification technique. *Nucleic Acids Res* 1992; **20**: 1691–1696.
13. Little MC, Andrews J, Moore R *et al.* Strand displacement amplification and homogeneous real-time detection incorporated in a second-generation DNA probe system, BDProbeTecET. *Clin Chem* 1999; **45**: 777–784.
14. Freeman R, Magee J, Barrett A. Identifying sputum specimens of high priority for examination by enhanced mycobacterial detection, identification, and susceptibility systems (EMDISS) to promote the rapid diagnosis of infectious pulmonary tuberculosis. *J Clin Pathol* 2001; **54**: 613–616.
15. Anon. Update: Nucleic acid amplification tests for tuberculosis. *MMWR Morb Mortal Wkly Rep* 2000; **49**: 593–594.
16. Down JA, O’Connell MA, Dey MS *et al.* Detection of *Mycobacterium tuberculosis* in respiratory specimens by strand displacement amplification of DNA. *J Clin Microbiol* 1996; **34**: 860–865.
17. Bergmann JS, Keating WE, Woods GL. Clinical evaluation of the BDProbeTec ET system for rapid detection of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2000; **38**: 863–865.
18. Somu N, Swaminathan S, Paramasivan CN *et al.* Value of bronchoalveolar lavage and gastric lavage in the diagnosis of pulmonary tuberculosis in children. *Tubercle Lung Dis* 1995; **76**: 295–299.