Short Communication

Preliminary study on rapid identification of *Mycobacterium tuberculosis* complex isolates by the BD ProbeTec ET system

S. X. Wang, L. H. Sng and L. Tay

Central Tuberculosis Laboratory, Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608

Correspondence
S. X. Wang
gptwsx@sgh.com.sg

Received 4 September 2003
Accepted 2 October 2003

The BD ProbeTec ET system for identification of *Mycobacterium tuberculosis* complex (MTBC) isolates from BACTEC 12B culture vials was evaluated in comparison with BACTEC NAP (*p*-nitro-α-acetylamino-β-hydroxy-propiophenone) differentiation. Of 145 mycobacterial isolates tested, comprising 89 MTBC and 56 non-tuberculous mycobacteria (NTM), BD ProbeTec ET correctly identified 87 MTBC and 56 NTM but missed two MTBC. Three NTM were misidentified when NAP was incubated at 37 °C only. Overall sensitivity, specificity and positive and negative predictive values were respectively 97·8, 100, 100 and 96·6 % for the BD ProbeTec ET system and 100, 94·6, 96·7 and 94·6 % for NAP.

Introduction

Tuberculosis (TB) remains a public health problem in many countries around the world. Although efforts have been made to eliminate this airborne disease, new cases of TB infection are still on the rise in some countries. According to a WHO report, there are 20 million cases of TB worldwide, with 8 million new cases and 3 million deaths each year. One of the most critical aspects of TB control is rapid identification of infectious patients (American Thoracic Society, 1997; WHO, 2003). Although there are commercially available kits for rapid detection of DNA of members of the *Mycobacterium tuberculosis* complex (MTBC), culture is still needed to confirm the presence of 'live' MTBC from a suspected patient's specimen as well as for drug susceptibility testing (Kent & Kubica, 1985; American Thoracic Society, 1997). The BACTEC radiometric method using the BACTEC460 TB system is one of the rapid methods used for culture of mycobacteria. Identification using BACTEC NAP (*p*-nitro-α-acetylamino-β-hydroxy-propiophenone) requires 4–8 working days to give a result. The BD ProbeTec ET system utilizes homogeneous strand displacement amplification technology as the amplification method and fluorescent energy transfer (ET) as the method of detecting the presence of MTBC from cultures (Spargo *et al.*, 1996; Bergmann & Woods, 1998; Little *et al.*, 1999). As the turnaround time for identification of MTBC isolates by the BD ProbeTec ET system is only 1 working day, use of the BD ProbeTec ET system can significantly reduce the time for identification. The objective of this study was to compare the sensitivity and specificity of the BD ProbeTec system with the existing NAP method for identification of MTBC isolates from BACTEC 12B culture vials.

Abbreviations: AFB, acid-fast bacilli; IAC, internal amplification control; MTBC, *Mycobacterium tuberculosis* complex; NAP, *p*-nitro-α-acetylamino-β-hydroxy-propiophenone; NTM, non-tuberculous mycobacterium; TB, tuberculosis.

Methods

Specimens used were 140 clinical specimens sent to our laboratory for mycobacterial culture and five mycobacteriology survey specimens from the College of American Pathologists (CAP 2000EB). Altogether, there were 103 respiratory specimens (92 sputum, five bronchial wash, three laryngeal swab and three gastric fluid) and 42 non-respiratory specimens (23 tissue, three pleural fluid, five wound swab, four pus, three blood, two urine and one each for cerebrospinal fluid and body fluid). Samples were processed by the NaOH digestion/decontamination procedure using 4 % NaOH (Petroff, 1915). After centrifugation, one or two drops of phenol red indication solution were added to each sediment, which was neutralized by adding 2 M HCl dropwise until the colour of the sediment turned from red to yellow. The sediment was then resuspended in 1 ml phosphate buffer (pH 6·8). An aliquot (0·5 ml) of processed specimen was inoculated into a BACTEC 12B culture vial supplemented with 0·1 ml antimicrobial mixture. Culture vials were then incubated at 37 °C. BACTEC 12B cultures showing a minimum growth index (GI) of $\geqslant$50 were investigated. An acid-fast bacilli (AFB) smear was performed and, if positive, the NAP identification test was carried out using a standard method (Siddiqi, 1995). NAP-differentiated MTBC isolates from non-respiratory specimens and MTBC isolates from respiratory specimens from patients without known MTBC isolation within the last 12 months were confirmed by a DNA probe for MTBC (AccuProbe). Each culture-positive isolate that was differentiated as a non-tuberculous mycobacterium (NTM) by NAP was subcultured on solid medium and identified to the species level using conventional biochemical tests and commercial DNA probes for *Mycobacterium kansasii*, *Mycobacterium avium* complex and *Mycobacterium gordonae* (AccuProbe).

Identification using the BD ProbeTec ET system (Becton Dickinson Diagnostic Instruments) was performed according to the manufacturer's instructions. Briefly, when the GI of a 12B vial reached 500 or above, 500 μl of a well-mixed culture was added to 1·0 ml wash buffer in a centrifuge tube, vortexed for 5 s and centrifuged at 12 200 ***g*** for 3 min. The pellet was heated for 30 min at 105 °C to render the organisms non-viable. After being pulse-centrifuged for 10 s, the pellet was resuspended in 100 μl lysis buffer, vortexed for 5 s and placed in a 65 °C sonic water bath (Branson Ultrasonic) for 45 min. The sonicated sample was pulse-centrifuged for 10 s, followed by addition of 600 μl neutralization buffer. The mixture was then vortexed for 5 s, pulse-centrifuged for 10 s and tested immediately. For each BD ProbeTec ET assay, one positive and one negative control were included. A known MTBC strain, ATCC 27294^{T}, was also included as a sample processing control. In addition, four clinical specimens (three pleural fluid, one lung biopsy) with negative AFB culture results were tested by BD ProbeTec ET assay. Using an eight-channel pipettor and aerosol-resistant tips, 150 μl of each sample or control was dispensed into a priming microwell. The priming microwell plate was covered and incubated at room temperature for 30 min before being placed into a 72·5 °C heating block. After incubation at 72·5 °C for 10 min, 100 μl from each priming microwell was transferred to the corresponding amplification microwell, which had been prewarmed in a 54 °C heating block for 10 min. The amplification microwells were then sealed and placed immediately in the BD ProbeTec ET instrument for 60 min. Samples with MTBC MOTA (metric other than acceleration) values greater than 7000 were considered positive for MTBC, regardless of the internal amplification control (IAC) MOTA. If the MTBC MOTA was less than 7000 and the IAC MOTA was greater than 5000, the specimen was considered negative for MTBC. If the MTBC MOTA was less than 7000 and the IAC MOTA was less than 5000, the result was considered indeterminate and the processed sample was tested again. If the NAP and BD ProbeTec ET System results were discordant, a frozen aliquot of the discrepant sample was tested again on the BD ProbeTec ET system and culture from BACTEC 12B vial was confirmed by DNA probe for MTBC.

Results and Discussion

Of the 145 positive AFB isolates studied, comprising 89 MTBC and 56 NTM (24 *M. avium* complex, 13 *M. kansasii*, five *M. gordonae*, three *Mycobacterium haemophilum*, two each of *Mycobacterium fortuitum* and *Mycobacterium chelonae*, one *Mycobacterium scrofulaceum* and six unidentifiable NTM), 87 MTBC and 56 NTM were identified correctly by BD ProbeTec ET. Three NTM from tissue samples were misidentified as MTBC by NAP initially while incubating the cultures at 37 °C (Table 1). Further investigation found that the three samples were best grown at approximately 30 °C and needed haemin to grow. A repeat NAP test on the cultures incubated at 30 °C identified the three samples as NTM, and final identification results suggested *M. haemophilum*. DNA probes for MTBC were negative for the three samples. Two MTBC were not detected by BD ProbeTec ET. One of the two was isolated from tissue and the other was from a laryngeal swab. The two MTBC cultures were retested by BD ProbeTec ET but showed reproducible negative results. MTBC and IAC MOTA values were respectively 144 and 21 157 and 134 and 55 372 for the tissue and laryngeal swab; as the IAC data showed no evidence of inhibition, these two specimens should be regarded as true false-negatives that might be due to mutation of the target gene. Cultures from the original BACTEC 12B vials were positive for MTBC by DNA probe. Overall sensitivity, specificity and positive and negative predictive values were respectively 97·8, 100, 100 and 96·6 % for BD ProbeTec ET and 100, 94·6, 96·7 and 94·6 % for NAP. Four clinical specimens (three pleural fluid, one lung biopsy) with negative AFB culture results tested by BD ProbeTec ET were negative.

Table 1. Identification of AFB-positive isolates by NAP and BD ProbeTec ET

Parameter	NAP	BD ProbeTec ET
Positive AFB isolates (n):		
MTBC ($n = 89$)	92*	87
NTM ($n = 56$)	53	56
Sensitivity (%)	100	97·8
Specificity (%)	94·6	100
Positive predictive value (%)	96·7	100
Negative predictive value (%)	94·6	96·6

*Including three MTBC isolates misidentified by NAP at 37 °C.

Mycobacterial culture using the BACTEC460 TB system is one of the rapid methods for isolation of MTBC from patients with suspected TB. The low-cost BACTEC NAP method, though outdated and with certain restrictions (Siddiqi, 1995), is still being used for differentiation of MTBC from NTM in some laboratories. As NAP alone is not good enough for identification of MTBC isolates, an equivalent molecular target approach such as Gen-Probe, Roche Amplicor PCR assay, ligase chain reaction or enhanced amplified direct test may be introduced for confirmation, especially for MTBC isolated from non-respiratory specimens and patients with unknown MTBC infection. The reported sensitivities for the molecular methods range from 87 to 100 %, and all achieve 100 % specificity (Reisner *et al.*, 1994; Smith *et al.*, 1997; Tortoli *et al.*, 1998; Bergmann & Woods, 1999). However, the high cost of performing molecular tests is a disadvantage. A few publications have recently reported that rapid diagnosis of MTBC in pulmonary and extrapulmonary specimens by the BD ProbeTec ET System showed sensitivity ranging from 77·8 to over 90 % and specificity of 99·5 to 99·8 % (Bergmann *et al.*, 2000; Piersimoni *et al.*, 2002; Mazzarelli *et al.*, 2003). As for culture confirmation, not enough study data were available. Our data showed that the BD ProbeTec ET system, which is from the manufacturer of BACTEC460 TB system, is a possible alternative method for rapid identification of MTBC from positive BACTEC vials. During the evaluation period, we found that the BD ProbeTec ET system is easy to use and can perform up to 96 identification tests in one run; sensitivity and specificity for differentiation of MTBC from NTM are satisfactory. The BD ProbeTec ET differentiation procedure is less labour-intensive and more efficient than NAP. Identification by the BD ProbeTec ET system can be completed within 1 working day, whereas NAP needs 4–8 days. All reagents used in the BD ProbeTec ET system may be stored at room temperature and, therefore, no refrigeration or freezing is required.

In summary, our study suggests that the BD ProbeTec ET system is reliable for identification of MTBC isolates in conjunction with the BACTEC460 TB system. It provides shorter turnaround times and better specificity than BACTEC NAP. However, as the number of isolates included in our study was small, it will be useful to conduct further studies to confirm our findings.

Acknowledgements

The authors wish to thank Becton Dickinson (Singapore) Pty Ltd for providing BD ProbeTec ET equipment and reagent kits and Ms Hla Hla Htay for her assistance during this study.

References

American Thoracic Society (1997). Rapid diagnostic tests for tuberculosis: what is the appropriate use? *Am J Respir Crit Care Med* **155**, 1804–1814.

Bergmann, J. S. & Woods, G. L. (1998). Clinical evaluation of the BDProbeTec strand displacement amplification assay for rapid diagnosis of tuberculosis. *J Clin Microbiol* **36**, 2766–2768.

Bergmann, J. S. & Woods, G. L. (1999). Enhanced amplified *Mycobacterium tuberculosis* direct test for detection of *Mycobacterium tuberculosis* complex in positive BACTEC 12B broth cultures of respiratory specimens. *J Clin Microbiol* **37**, 2099–2101.

Bergmann, J. S., Keating, W. E. & Woods, G. L. (2000). Clinical evaluation of the BDProbeTec ET system for rapid detection of *Mycobacterium tuberculosis*. *J Clin Microbiol* **38**, 863–865.

Kent, P. T. & Kubica, G. P. (1985). *Public Health Mycobacteriology: a Guide for the Level III Laboratory*. Atlanta, GA: Centers for Disease Control, US Department of Health and Human Services.

Little, M. C., Andrews, J., Moore, R. & 22 other authors (1999). Strand displacement amplification and homogeneous real-time detection incorporated in a second-generation DNA probe system, BDProbeTec ET. *Clin Chem* **45**, 777–784.

Mazzarelli, G., Rindi, L., Piccoli, P., Scarparo, C., Garzelli, C. & Tortoli, E. (2003). Evaluation of the BDProbeTec ET system for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary samples: a multicenter study. *J Clin Microbiol* **41**, 1779–1782.

Petroff, S. A. (1915). A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. *J Exp Med* **21**, 38–42.

Piersimoni, C., Scarparo, C., Piccoli, P., Rigon, A., Ruggiero, G., Nista, D. & Bornigia, S. (2002). Performance assessment of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex from respiratory and extrapulmonary specimens. *J Clin Microbiol* **40**, 4138–4142.

Reisner, B. S., Gatson, A. M. & Woods, G. L. (1994). Use of Gen-Probe AccuProbes to identify *Mycobacterium avium* complex, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae* directly from BACTEC TB broth cultures. *J Clin Microbiol* **32**, 2995–2998.

Siddiqi, S. H. (1995). *BACTEC460 TB System, Products and Procedure Manual*, revision D. Sparks, MD: Becton Dickinson.

Smith, M. B., Bergmann, J. S. & & Woods, G. L. (1997). Detection of *Mycobacterium tuberculosis* in BACTEC 12B broth cultures by the Roche Amplicor PCR assay. *J Clin Microbiol* **35**, 900–902.

Spargo, C. A., Fraiser, M. S., Van Cleve, M., Wright, D. J., Nycz, C. M., Spears, P. A. & Walker, G. T. (1996). Detection of *M. tuberculosis* DNA using thermophilic strand displacement amplification. *Mol Cell Probes* **10**, 247–256.

Tortoli, E., Lavinia, F. & Simonetti, M. T. (1998). Early detection of *Mycobacterium tuberculosis* in BACTEC cultures by ligase chain reaction. *J Clin Microbiol* **36**, 2791–2792.

WHO (2003). *WHO report 2003. Global tuberculosis control*. Geneva: World Health Organization. http://www.who.int/gtb/publications/globrep/index.html