Full Length Research Paper

Evaluation of the BD MGIT™ TBc identification test for rapid identification of Mycobacterium tuberculosis Complex from positive BACTEC MGIT 960 cultures in a routine laboratory work

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A new immunochromatographic assay BD MGIT™ TBc identification test for rapid identification of Mycobacterium tuberculosis from BACTEC MGIT 960 positive cultures was evaluated. The study tested 273 positive MGIT 960 cultures with TBc ID test. The results of the test were compared with conventional biochemical tests as gold standard. The sensitivity, specificity, and positive/negative predictive value of TBc ID test for identifying M. tuberculosis complex were 96.9, 100, 100 and 88.9% respectively. We conclude that the test is very useful and specific tool for rapid identification of M. tuberculosis complex from positive MGIT 960 cultures and the test can be used in a routine laboratory.

Key words: Mycobacterium tuberculosis complex, BACTEC MGIT 960 system, identification, immunochromatographic test.

INTRODUCTION

During recent years the prevalence of tuberculosis (TB) and non tuberculous mycobacterial (NTM) infections have increased in the world, mainly as a consequence of several factors such as the AIDS epidemic. Mycobacterium tuberculosis complex (MTBC), the causative agent of most TB cases is responsible for more than 1.1 million deaths and 8.8 million new cases of disease every year (WHO, 2010). On the other hand, NTM are a major cause of opportunistic infections in immunocompromised patients and patients with chronic lung diseases (Horsburgh and Selik, 1989). The clinical characteristics of NTM-related pulmonary disease are in many cases extremely similar to those of TB (Falkingham, 1996). Although, there is very little information about diseases associated with NTM in developing countries. However, in recent studies in Zambia and South Africa using better diagnostic tools have actually reported a high prevalence of NTM infections in HIV/AIDS patients (Pettipher et al., 2001; Buijtels et al., 2009). For this reason, rapid detection and identification of the type of mycobacteria are extremely important for optimal diagnosis and effective treatment, as well as for prevention and control of TB transmission.

World health organization (WHO) recommends the use of liquid culture systems for rapid detection and drug susceptibility tests as a standard method for TB diagnosis and case management (WHO, 2007). Nonradiometric culture systems such as BD BACTEC™ MGIT™ system, can detect mycobacteria in shorter time than solid media (Lowenstein-Jensen media) (Katila et al., 2000; Lee et al., 2003). Although this system cannot differentiate between MTBC and NTM in positive results and confirmatory testing should be performed by subculture on solid medium and biochemical tests. Conventional biochemical tests can be used to identify Mycobacteria species; but they are troublesome, time-consuming and laborious. These tests may require turnaround time of 6
to 9 weeks or longer. Other methods, such as nucleic acid amplification and high-performance chromatography are now available for species identification of mycobacteria (Telenti et al., 1993; Suffys et al., 2001; Pinsky and Banaei, 2008; Toney et al., 2010). These methods have been applied in the clinical laboratories with great success. However, some of these methods require trained personnel and special laboratory. They are expensive and labour-intensive for routine clinical use in resource-limited countries. Therefore, there is an urgent need to develop rapid and simple test to perform accurate identification.

Recently, BD (Becton Dickinson, USA) developed a simple immunochromatographic assay BD MGIT™ TBc ID test (TBc ID test) using anti-MPT 64 antibodies for rapid differentiation between MTBC and NTM in as little as 15 min. The immunogenic protein MPT 64 has been shown to be specifically secreted by *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* and some but not all substrains of *M. bovis* BCG (Nagai et al., 1991; Abe et al., 1999; Reddy et al., 2002) and can be detected in culture isolates. Anti-MPT 64 antibodies were immobilized on a nitrocellulose membrane as the capture material. Secondary antibody, which recognized another epitope of the MPT64 antigen, conjugated with colloidal gold particles, was used for antigen capture and detection in a sandwich-type assay.

The purpose of this study was to evaluate the performance of the TBc ID test for rapid detection and identification of MTBC, directly from positive MGIT 960 cultures and the results were compared with conventional biochemical tests as gold standard.

The study was performed in TB-Laboratory at National Center for Diseases Control from January to December 2010. During this period, we received 1959 specimens (three consecutive sputum, tracheal aspirate, bronchial washing and bronchoalveolar fluid) from patients with suspected TB. All specimens were processed inside class II biological safety cabinet for detection of *Mycobacterium* spp. by direct microscopy (Ziehl-Neelsen staining) and routine mycobacterial culture. Of these specimens, 82 were smeared positive for acid-fast bacilli (AFB). The specimens were decontaminated by the N-acetyl-L-cystine-NaOH (Mycob prep- Becton Dickinson) method according to the standard procedure as recommended by the manufacturer. For mycobacterial culture, 500 µl of each decontaminated specimens was inoculated into an MGIT culture tube containing both 10% OADC (oleic acid, albumin, dextrose, and catalase; Becton Dickinson) and 0.8 ml of PANTA antimicrobial supplement (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin; Becton Dickinson), as per manufacturer’s instructions. Inoculated MGIT tubes were incubated in the MGIT culture system at 37°C and examined daily for 42 day. All positive MGIT culture tube were inoculated on Lowenstein-Jensen medium and incubated at 37°C for 8 weeks or until mycobacterial colonies were seen. Isolates of mycobacteria were identified to the species level by their growth rate, colony morphology, pigmentation and conventional biochemical tests, such as the para-nitrobenzoic acid (PNB), thiophen-2-carboxylic acid hydrazide (TCH), niacin test, nitrate reduction test, urease, semi-quantitative catalase, Tween 80 hydrolysis, NaCl tolerance (Leao et al., 2004).

TBc ID test was performed according to the manufacturer. 150 µ of positive liquid culture fluid was placed into the TBc ID slide test well without any manipulation and the test was read after 15 minute. The appearance of two pink-red lines, one in test zone (T) and other in control zone (C) indicated a positive result for MTBC, whereas the presence of a control zone (C) alone indicated a negative result for MTBC. If the control zone (C) was not visible after 15 minute, the result was considered invalid, and the sample was retested. A color line of any intensity was read as a positive reaction (Figure 1).

Of the total 273 specimens with positive results by MGIT 960 cultures system, 125 specimens (45.8%) were positive for MTBC and 148 specimens (54.2%) were negative for MTBC by TBc ID test. Among the isolates with negative results, 112 isolates were contaminated with unidentified species of fungi and bacteria, whereas 32 isolates were grown on solid medium and identified as NTM by biochemical tests (Table 1). The most prominent NTM species identified by biochemical tests were *Mycobacterium avium* complex, *Mycobacterium kansasii*, *Mycobacterium fortuitum*, *Mycobacterium chelonae*. Using standard biochemical tests as a reference, the sensitivity, specificity, positive predictive value and negative predictive value of the TBc ID test for identification of MTBC in the positive MGIT cultures were 96.9, 100, 100 and 88.9% respectively.

In this study, 4 isolates were negative used the TBc ID test, which were confirmed to contain MTBC by biochemical testing. These false negative results also were repeated by TBc ID test from colonies taken from solid media. 3 of these isolates yielded positive results, while one isolate yielded negative result. According to the previous studies, mutations and deletions on MPT64 gene were observed in some strains of MTBC (Hirano et al., 2004). However, these mutations may lead to false negative TBc ID test result. Because the DNA analysis was not performed in our isolates, we cannot be sure if the negative results were related to mutation in *mpt* 64 gene. Another possible explanation for the false negative results is the low concentration of MTP64 protein on the first culture-positive day. Study by Lee et al. (2010), observed the performance of immune-chromatographic assay from positive MGIT culture on the seventh day was higher than that on the first culture-positive day.

The results of our study show that the TBc ID test is a reliable test for rapid identification of MTBC in BACTEC MGIT 960 culture system. The median time from specimen received in the laboratory and identification of
Figure 1. Identification of *Mycobacterium tuberculosis* complex by ID TBC test from BACTEC MGIT 960 positive cultures. Left, positive result (strong); Middle, positive result (weak); Right, negative result.

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<th>Table 1. Results of BD MGIT™ Tbc ID test compared with biochemical tests.</th>
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<td><strong>Tbc ID test result</strong></td>
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<td>Positive</td>
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<td>Negative</td>
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*These isolates were considered as contaminated cultures.

MTBC was 21 days (range, 4 to 42 days) for Tbc ID test compared with 45 days (range, 30 to 75 days) for biochemical tests. Infections due to NTM have been reported to account for 0.5 to 30% of all mycobacteria infections (Wallace et al., 1990). Of all mycobacteria isolated from our patients, 80% were MTC, whereas 20% were NTM. However, it is very important to isolate and identify mycobacteria species, especially MTBC for clinical management and control of TB transmission. Recently, various immune-chromatography assay based to detect MTB 64 antigen have been introduced for rapid identification of MTBC in liquid culture system, such as Capilia TB assay (Tauns Laboratories, Inc. Numazu, Japan), The Tibilia rapid test (Hangzhou, china), The SD Bioline TB Ag MPT 64 rapid test (Standard Diagnostics, South Korea), and the BD MGIT™ Tbc ID test. All of these assays demonstrated excellent sensitivity and specificity in identifying the MTBC (Park et al., 2009; Ngeow et al., 2011; Yu et al., 2011).

In conclusion the Tbc ID test is a useful test for identifying MTBC from positive MGIT culture medium. The test could be implemented in any mycobacteriology laboratory in order to accelerate time to diagnose TB, especially in resource limited countries, where the implementation and usage of molecular methods is often not possible.

REFERENCES


Hirano K, Aono A, Takahashi M, Abe C (2004). Mutations including


