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Utility and diagnostic performance of *Mycobacterium tuberculosis* complex by two immunochromatographic assays as compared with the molecular Genotype assay in Nigeria

Benjamin Thumamo Pokam [a,*], *Anne E. Asuquo* [b], *Khye Seng Goh* [c], *Lydia N. Abia-Bassey* [b], *Nalin Rastogi* [c]

[a] *Department of Medical Laboratory Science, Faculty of Health Sciences, University of Buea, Cameroon*

[b] *Department of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Nigeria*

[c] *WHO Supranational TB Reference Laboratory, TB & Mycobacteria Unit, Institut Pasteur de la Guadeloupe, 97183 Abymes, Guadeloupe, France*

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ABSTRACT

Among the disadvantages of smear microscopy for detection of tuberculosis cases is its inability to differentiate between *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacteria (NTM). This study evaluated two, new immunochromatographic assays – Capilia TB-Neo and SD Bioline – on unheated and heated cultures at 80 °C for 30 min respectively for their ability to discriminate between MTB complex and NTM as compared with the molecular Genotype assay. Mycobacteria used in the study were obtained from smear-positive specimens collected from patients at four major hospitals in Cross River State, Nigeria. Capilia TB-Neo and SD Bioline showed sensitivities of 98.8% and 93.8% respectively and 100% specificity for both assays. Heating the isolates did not significantly impact the test performance. Both tests are recommended for use in rapid differentiation of strains isolated in Nigeria.

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Introduction

The rise in HIV infections and the neglect of tuberculosis (TB) control programs have enabled the resurgence of TB [1]. Tuberculosis caused by *Mycobacterium tuberculosis* [MTB] is a major disease of great public health concern. There were 9.4 million new TB cases in 2009, including 1.1 million cases among people with HIV (http://www.who.int/tb/publications/2010/factsheet_tb_2010.pdf). The highest incidence rates in Africa are in South Africa and Nigeria, with the latter having an estimated 460,000 new cases per year [2]. Basic diagnosis of TB has not changed for more than a century; therefore, finding simple, quick tests is a priority and remains a major challenge to fighting TB (http://www.stoptb.org/resources/factsheets/fastfacts.asp).

Although in regions where TB is highly prevalent as in Nigeria, most culture-positive mycobacteria are of the MTB complex. Globally, non-tuberculous mycobacteria (NTM) isolates have been increasing gradually [3]. NTM trigger diseases and true infections and thus can be important clinically [4]. Data on NTM disease in sub-Saharan Africa are limited, owing mainly to the lack of culture facilities, as well as the complexity of identification methods for mycobacterial species. As a result, many laboratories do not discriminate between MTB and NTM [5–7], and treatment in most African countries, including Nigeria, relies on sputum smear microscopy. The

* Corresponding author. Address: Department of Medical Laboratory Science, Faculty of Health Sciences, University of Buea, P.O. Box 63, South West Region, Buea, Cameroon. Tel.: +237 77358743.

E-mail address: thumamo@yahoo.fr (B.T. Pokam).

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implication is that NTM is inappropriately managed with first-line anti-tuberculous drugs [7,8], thereby worsening the patient's condition and increasing the risk of drug resistance.

The acid-fast bacterium is known to produce 200 or more kinds of protein, and MTB has been known to secrete more than 33 different proteins [9]. The mycobacterial antigen MPB64, isolated from culture filtrates of *Mycobacterium bovis* BCG since 1984, and detected by the immunochromatographic method was later confirmed by gene-based analysis to be identical to MPT64 produced by MTB. NTMs do not produce MPB64. This secreted mycobacterial protein has been used for diagnostic purposes, and is found in unheated culture media of MTB, *M. bovis*, and some but not all substrains of *M. bovis* BCG [10–15].

In an attempt to assess the sensitivities and specificities of two immunochromatographic techniques in the differentiation of MTB and NTM and their usage on strains isolated in Nigeria, Capilia TB-Neo and SD Bioline kits were used on both unheated and heat-killed cultures, respectively, and compared with the Genotype assay.

Materials and methods

The study was carried out in Cross River State, located in the southern region of Nigeria. Subjects included 97 sputum smear-positive patients of both sexes, from all age groups seen in the major hospitals and TB care facilities of the northern, central and southern senatorial districts of the State. Sputum specimens obtained from patients were preserved using sodium carbonate (75 mg), and refrigerated until cultured.

Specimens were decontaminated using the modified Petroff method, and cultured using BACTEC 960. Smears were made from isolates obtained from the BACTEC MGIT tubes, stained by the Ziehl Neelsen staining method, and examined for the presence of Acid Fast Bacilli (AFB).

The growth on AFB positive MGIT tubes was further inoculated into the Lowenstein–Jensen slants where pure cultures were obtained. Recovery of the organisms and identification using Capilia TB-Neo (TAUNS Laboratories, Inc., Japan) was performed according to manufacturer's instructions (http://capilia.jp/english/capilia_tb_neo.html).

A turbid suspension for each isolate was prepared using 1 ml of distilled water and approximately 3 loopsful of bacteria from Lowenstein–Jensen media, The bacteria were heat-killed in a water bath at 80 °C for 30 min, preserved at −80 °C until tested using SD Bioline according to manufacturer's instructions (Standard Diagnostics, Inc., Yongin, Korea).

To determine the species of the isolates, the Genotype Mycobacterium CM kit was used according to manufacturer's instructions (Hain Lifescience GmbH, Nehren, Germany).

Results

A total of 97 strains were isolated on Lowenstein–Jensen medium and tested for the presence of the mycobacterial protein using both kits; 76 were positive for both Capilia and SD Bioline, while 17 were negative. On the other hand, 4 SD Bioline negative isolates were Capilia positive, bringing Capilia positive isolates total to 80 (Table 1). Further identification of all the isolates was carried out using Hain (Table 2). Eighty Capilia positive isolates were identified as members of the MTB complex, and one isolate of the 17 Capilia negative was also identified as a member of the MTB complex (Sensitivity = 98.8%). On the other hand, 5 SD Bioline negative isolates were identified as members of the MTB complex (Sensitivity = 93.8%). No false positive results were obtained using both tests (Specificity = 100%).

Discussion

This study has shown a sensitivity of 98.8% and 93.8% for Capilia TB-Neo and SD Bioline respectively, and 100% specificity for both assays. Some evaluation studies have reported sensitivity and specificity of 99.2% and 100% for Capilia TB assay, which is in accordance with this study, and thus useful in rapid differentiation of MTB complex from NTM [10,16]. Another study has shown lower sensitivity (92.4%) with Capilia TB kit [17]. No false positive was observed in this study as reported in earlier studies [16,17].

Although most strains of MTB complex are usually correctly identified with Capilia, some Capilia TB negative strains have been isolated elsewhere [18]. In this study, the Capilia TB negative and SD Bioline negative isolate was later identified by Genotype Mycobacterium CM/spoligotyping tests as MTB complex/ *Mycobacterium africanum* [19]. The failure of Capilia TB to detect the MPB64 has been previously ascribed to mutation within the MPB64 gene that led to the production of an incomplete protein as a result of a deletion of the C-terminal region of the protein [18]. Although this study did not investigate the reason for this observation, a similar occurrence may be responsible.

The SD Bioline test is very accurate in detecting MPT64 antigen, although the manufacturer has recognized a low incidence of false negative results (Sensitivity of 98.6%). The SD Bioline sensitivity of 93.8% in this study may be partly due to denaturation of the MPT 64 protein following the application of heat to kill the isolates prior to testing and partly to the use of distilled water rather than the buffer recommended by the manufacturer. Recent evaluation studies have reported excellent sensitivities of 100% [20,21], 99% [22,23], and 97.1% [24]. As in this study, several studies have reported 100% specificity [20–24].

The 93.8% sensitivity of this study, despite heating of isolates and use of water rather than extraction buffer indicate that the test performance was not greatly influenced by these factors. The isolates were heat-killed prior to their transport to Guadeloupe for molecular analysis.

Capilia and SD Bioline may well be adapted in the identification of mycobacterial strains isolated in Nigeria, although further studies using Capilia on heat-killed organisms are required. This study confirmed that the methods are fast and easy to perform with high sensitivity and specificity. These methods present as more rapid and easier alternatives for identification of MTB Complex isolates compared with the more cumbersome traditional ones, such as Niacin, Nitrate and 68 °C Catalase, still currently used in Nigeria.

Although immunochromatographic methods can ascertain that an isolate is a member of the TB complex (sensitiv-

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