EVALUATION OF DIASPOT TUBERCULOSIS RAPID TEST FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN ABIA STATE, NIGERIA

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ABSTRACT

Lack of simple, rapid and accurate diagnostic tests is currently one of the major constraints hindering effective control of Tuberculosis (TB) in developing countries. Sputum smear microscopy, the most widely used method, has a problem of low sensitivity and other shortcomings that limit its quality and scope of application. Rapid Serological TB antibody detection (immunochromatographic) technique represents the new generation of rapid diagnostic tests (RDTs) with greatest potential impact if they are found accurate enough for diagnosis of TB in endemic countries. The objective of the study reported here was to evaluate the performance of an immunochromatographic assay, DiaSpot TB Rapid test, for diagnosis of pulmonary tuberculosis in Abia State, Nigeria. Sera from 194 TB suspects were tested by DiaSpot TB Rapid test. The sensitivity and specificity of the test for diagnosis of PTB were 39.2% (95% Confidence Interval [CI]): 28.4-50.0) and 85.4 (95% CI: 77.6-93.2) respectively against a reference standard of sputum culture on Lowenstein-Jensen medium. The positive and negative predictive values of the rapid test were 83.8% (95% CI: 75.7-91.9) and 42.2 (95% CI: 31.3-51.3) respectively. Our results showed that although DiaSpot TB Rapid test had an acceptable level of specificity, the sensitivity was low and the test did not perform as well as sputum smear microscopy. However, the sensitivity improved when the results were combined with that of sputum smear microscopy, suggesting that the test may be useful as a supplement to microscopic method.

Key words: Pulmonary tuberculosis, serological tests, rapid TB test

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INTRODUCTION

The current global Tuberculosis (TB) epidemic causes an estimated 9 million cases of active disease and 2 million deaths worldwide every year making TB a leading global public health problem (Kherad et al, 2009, Dye, 2006, WHO/TDR, 2006a). TB is a major public health problem in Nigeria. Nigeria recently moved from the 4th to the 5th position of the 22 countries with highest burden of TB in the World (FMOH, 2008, WHO, 2007). The internationally recommended strategy for control of TB is the directly observed treatment short course (DOTS). Accurate and early case detection of TB is the cornerstone of the success of this strategy (Perkins and Kritski, 2002). Sputum smear microscopy, the current most widely used method for diagnosis of TB in areas where TB is endemic, including Nigeria, has several limitations. In particular, the test has inherent problems of low and variable sensitivity (Young et al, 2008, Steingart et al, 2007, Siddiqi et al., 2003); is not good at detecting early disease (Perkins, 2000); requires considerable technical training (Keeler et al, 2006). The procedure for sputum smear microscopy is labour- intensive, time consuming and highly inconvenient for patients who must make multiple visits to the laboratory to submit specimens and collect results (Perkins et al., 2006, Mase et al, 2007, Squire et al, 2005). Furthermore, due to lack of infrastructure and personnel needed for establishment and operation of sputum
smear microscopy in rural areas in developing countries, the method has proved surprisingly difficult to implement in these countries (WHO/TDR, 2006b). A vast majority of TB patients are therefore cut off from access to diagnostic services except they can afford the travelling costs to microscopy centres typically located in urban areas. For these reasons simpler, more rapid and accurate TB tests are urgently needed in developing countries.

Rapid diagnostic tests (RDTs) that detect the presence of specific antibodies directed against immunodominant antigens of infectious agents have been developed. These antibody detection (serological) tests in simple rapid immunochromatographic format have greatly simplified the diagnosis of many infectious diseases (WHO/TDR, 2006a). Such tests have been greatly desired for TB for a long time (Daniel, 1988, Daniel and Debanne, 1987, Perkins et al., 2006). In pursuit of this objective, new rapid diagnostic tests for TB have been recently developed. Several of these tests have been evaluated in various studies in different parts of the world (WHO/TDR, 2008, Steingart et al, 2009). A recent systematic review of the past studies showed that the performances of the tests varied widely in different studies (Steingart et al, 2007). There is therefore a need for independent evaluation of TB serological antibody detection tests in particular epidemiological settings where they are intended for use so as to assess their diagnostic performance characteristics relevant to the local settings. In this paper, we report the prospective evaluation of DiaSpot TB Rapid test in Abia State.

MATERIALS AND METHODS
Study Participants. Between November 2008 and February 2010, TB suspects, defined as patients with cough of 3 weeks’ duration or more were recruited from the Leprosy and Tuberculosis Referral Hospital, Uzuakoli, Bende Local Government Area (LGA) and the Sputum Smear Microscopy Centre, Aba South LGA Health Office, Aba, two major TB control centres in Abia State. Eligible participants were TB suspects not less than 15 years newly referred to the study centres by a physician to undergo Sputum smear microscopy examination for Acid-fast bacilli (AFB). Those already on anti-tuberculosis treatment at the time of recruitment were excluded from the study. Informed consent was obtained from the study participants and the study protocol was approved by the research Ethical Committee of the Federal Medical Centre, Umuahia.

The TB suspects were instructed by to submit three sputum samples to the diagnostic centre according to the routine TB diagnostic process in the Abia State TB Control Programme. The first sample was collected on the spot the first day the patient visited the diagnostic centre. A container was given to the patient for collecting an early morning sample at home on the next day, and the third was collected on the spot when the patient brought the early morning specimen. TB suspects were screened for HIV on a voluntary basis as part of the routine laboratory investigation for TB under the Abia State TB Control Programme. Finger prick blood was usually used but for the purpose of this research project, all eligible subjects who gave informed consent were requested to give venous blood sample for both HIV testing and TB serology. The blood samples were collected into anticoagulant free plastic tubes from which the sera were later separated.

Acid-fast staining. A direct smear was made from each sputum specimen, stained by the ZN method, and read at the study centres by experienced laboratory technicians. A suspect was diagnosed as a smear-positive TB patient if at least one of the three smears was positive for AFB. Sputum culture. The sputum samples were transported to the
Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike (MOUAU) for processing and culture. The sputum samples were decontaminated and concentrated using modified Petroff’s alkali method (Cruickshank et al, 1975). Briefly, sputum was mixed with equal volume of 4% Sodium Hydroxide (NaOH). The mixture was placed in an incubator at 37°C for 30 minutes; and gently shaken at about every 5 minutes. About 15ml portion of the mixture was transferred into sterile plastic tubes and centrifuged at 3,000 rpm for 30 minutes. The supernatant was poured off and a drop of 1% phenol red solution was added to the deposit. The deposit was neutralized with 8% Hydrochloric acid; added drop-wise until the deposit turned from red through yellow to orange pink. About 0.2ml of processed specimen was inoculated unto slants of Lowenstein-Jensen (LJ) medium. The inoculated LJ slants were incubated at 37°C and examined for growth daily for the first 1 week and once weekly thereafter up to 8 weeks. Cultures that showed no growth after weeks were scored as negative. A patient was defined as true TB patient if the culture produced *Mycobacterium tuberculosis* and as a non-TB case if the culture showed no growth. Patients whose samples grew nontuberculosis mycobacteria (NTM) were regarded as culture-positive NTM patients. Contaminated cultures were so classified and excluded from analysis. The identity of *Mycobacterium* was made on the basis of rate of growth, culture pigmentation and colonial morphology.

DiaSpot Tuberculosis Rapid Test. The blood samples were transported to the Microbiology Laboratory, MOUAU, and the serum separated on the same day by centrifugation at 3,000rpm for 10 minutes. The sera were separated into serum sample containers using a separate pipette for each sample. The sera were used for testing the DiaSpot TB Rapid test. DiaSpot TB Rapid Test Device (whole blood/serum/plasma) is a rapid chromatographic two-site sandwich immunoassay for qualitative detection of anti-TB IgG, IgM and IgA antibodies in whole blood, serum or plasma specimens. The test utilizes a combination of recombinant antigens pre-coated on a nitrocellulose membrane strip at the test line to detect elevated levels of anti-TB antibodies in the specimens. The test method employs colloidal gold particles labeled with anti-human IgG as conjugates for visualization of the antibody-antigen reactions at the test lines. During testing the specimen is applied to the sample site where it mixes with the antibody coated particles. The mixture migrates upward on the membrane chromatographically by capillary action. If anti-TB antibodies are present in the specimen, they react with the immobilized TB recombinant antigens at the test line on the membrane. The reaction produces a coloured line. The presence of this coloured line in the test region indicates a positive result while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. The test device was removed from the sealed foil pouch, placed on a flat surface. Three drops of serum (approximately 75μl) was transferred to the specimen well (S) of the test device using the plastic dropper provided in the kit. The result was read at 10 minutes according to the manufacturer’s instructions.

HIV Screening: The study participants were screened for HIV using two rapid HIV tests according to the National HIV Screening guidelines. A patient was screened first with Determine™ HIV 1& 2 (manufactured by
Abbot Japan Co., Ltd for Inverness Medical, Japan Co., Ltd). If positive, the specimen was retested with DoublecheckGold™ Ultra HIV 1& 2 (Organics Ltd, Israel) for confirmation. The two tests must be positive for a patient to be regarded as HIV positive.

**RESULTS**

DiaSpot TB Rapid test was compared with smear microscopy in 194 suspected pulmonary Tuberculosis (PTB) patients. Sixty five (33.6%) of the patients were smear-positive for AFB. DiaSpot TB Rapid test was positive in 31 (47.7%) of the 65 smear-positive cases and in 26 (20.2%) of the 129 smear-negative cases. In total the rapid TB test was positive for anti-TB antibodies in 57 (31.7%) of the study population. The test produced invalid results in four patients due to failure of migration of the specimens on the test membrane. The ability of the test to detect the smear – positive cases according to AFB grading is shown in Table 1. Generally, the test appears to detect higher number of smear-positive cases but detection pattern did not correlate with quantitative AFB grades of the smear-positive cases (Table 1). To assess the sensitivity and specificity of DiaSpot TB Rapid test, the sputum samples of 150 patients were cultured for *M. tuberculosis* and the sera of the patients tested with DiaSpot TB Rapid test to detect anti-TB antibodies. *Mycobacterium* species were isolated from a total of 91 patients; 79 (86.8%) were identified as *M. tuberculosis* complex on the basis of rate of growth, colonial morphology and biochemical tests. A total of 18 (12%) of the cultures were contaminated. The ability of the DiaSpot TB test to identify the culture positive PTB cases is shown in Table 2. In 37 culture and smear positive cases, the test was positive in 20 (54.1%) and in 11 (26.2%) of 42 culture positive but smear-negative PTB cases. The over all performance of the test against a reference standard of sputum culture is presented in Table 3. The sensitivity and specificity were 39.24% and 85.4% respectively. And the positive and negative predictive values were 83.8% and 42.2% respectively. The specificity was reduced to 81.1% and positive predictive values to 75.6% when the nontuberculosis mycobacterial (NTM) isolates were included in the analysis. We combined the results of DiaSpot TB with those of smear microscopy to assess the possibility of using the test together. This resulted in improvement of the sensitivity from 39.2% to 60.5% but with a corresponding reduction in specificity from 85.4% to 58.5%. Table 4 shows the comparison of the performance characteristics of smear microscopy and the rapid TB test. The DiaSpot TB was not as sensitive but more specific than smear microscopy. There was a significant difference between the ability of the DiaSpot TB Rapid test and smear microscopy to detect culture positive pulmonary tuberculosis in the study population ($\chi^2 = 12.23$, $p<0.05$).

Out of a total of 184 participants who gave consent for HIV screening, 39(21.2%) were sero-positive for immunodeficiency virus (HIV) infection. The prevalence of HIV/TB coinfection was 30.77% (12/39). The DiaSpot TB test was positive in 4(33.3%) of this subgroup and smear microscopy was positive in 3(25%) but the two tests detected different patients. 

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Table 1: Performance of DiaSpot TB Rapid test in sputum smear positive PTB patients in Abia State

<table>
<thead>
<tr>
<th>AFB Grade</th>
<th>No. (%) Positive by SSM</th>
<th>No. (%) Positive by DiaSpot TB test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanty (1-9)</td>
<td>7/69 (10.14)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>1+ (10-99)</td>
<td>23/69 (33.33)</td>
<td>11/23 (47.83)</td>
</tr>
<tr>
<td>2+ (1-10)</td>
<td>19/69 (27.53)</td>
<td>11/19 (57.89)</td>
</tr>
<tr>
<td>3+ (&gt;10)</td>
<td>20/69 (28.99)</td>
<td>9/20 (45.00)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>69 (100)</td>
<td>31/69 (44.93)</td>
</tr>
</tbody>
</table>

*a* 1-9 AFB/100HPF (high power field)  
*b* 10-99 AFB/100HPF  
*c* 1-10 AFB/HPF  
*d* >10 AFB/HPF

Table 2. Detection of culture positive PTB cases by DiaSpot TB test in the study population

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>No. of cases</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>No. (%) with low signal (Faint) result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive, smear positive (Cx +ve, Sm +ve)</td>
<td>37</td>
<td>20 (54.05)</td>
<td>17 (45.95)</td>
<td>5 (25.00)</td>
</tr>
<tr>
<td>Culture positive, smear negative (Cx +ve, Sm -ve)</td>
<td>42</td>
<td>11 (26.19)</td>
<td>31 (73.81)</td>
<td>2 (20.00)</td>
</tr>
<tr>
<td>Culture positive, smear positive (NTM)*</td>
<td>8</td>
<td>2 (25.00)</td>
<td>6 (75.00)</td>
<td>1 (50.00)</td>
</tr>
<tr>
<td>Culture positive, smear negative (NTM)</td>
<td>4</td>
<td>2 (50.00)</td>
<td>2 (50.00)</td>
<td>1 (25.00)</td>
</tr>
</tbody>
</table>

*NTM - Nontuberculosis mycobacteria*
Table 3. Sensitivity, Specificity, PPV and NPV of DiaSpot TB in reference to sputum culture

<table>
<thead>
<tr>
<th>Diagnostic tests</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiaSpot TB</td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td>37</td>
<td>39.2 (28.4-50.0)(^a)</td>
<td>85.4 (77.6-93.2)</td>
<td>83.8 (75.7-91.9)</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48</td>
<td>35</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>35</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>41</td>
<td>120</td>
<td></td>
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</tr>
</tbody>
</table>

\(^a\)95% CI- 95% Confidence Interval  
PPV- Positive Predictive value  
NPV- Negative Predictive Value
Table 4: Diagnostic performance characteristics of sputum microscopy and DiaSpot TB Rapid test for diagnosis of PTB in Abia State

<table>
<thead>
<tr>
<th>Diagnostic tests</th>
<th>Total</th>
<th>No. of Samples</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True Positive</strong></td>
<td><strong>False Positive</strong></td>
<td><strong>True Negative</strong></td>
<td><strong>False Negative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum Microscopy (SSM)</td>
<td>132</td>
<td>37</td>
<td>12</td>
<td>41</td>
<td>42</td>
<td>46.8 (35.8-57.8)</td>
</tr>
<tr>
<td>DiaSpot TB test</td>
<td>132</td>
<td>31</td>
<td>10</td>
<td>43</td>
<td>48</td>
<td>39.2 (28.4-50.0)</td>
</tr>
<tr>
<td>SSM + DiaSpot TB test</td>
<td>132</td>
<td>48</td>
<td>22</td>
<td>31</td>
<td>31</td>
<td>60.8 (50.0-71.5)</td>
</tr>
</tbody>
</table>
DISCUSSION

We found a sensitivity of 39.2% and a specificity of 85.4% for DiaSpot TB Rapid test in this study as against the manufacturer’s reported sensitivity of 83.0% and 98.9%. Other investigators have reported low sensitivities for TB rapid tests in various studies. Ongut et al. (2006) reported a sensitivity of 33.3% and a specificity of 100% for ICT Tuberculosis test in Antalya, Turkey. Kassa-Kelembo et al. (2006) reported a poor performance for SDHO MTB test, with a sensitivity of 20.6% and a specificity of 90.3% in Bangui, Central African Republic. In a laboratory-based evaluation of 19 commercial TB serological tests by TDR researchers, sensitivities ranged from 0.97% to 59.7% and specificities ranged from 53% to 98.7% (WHO/TDR, 2009). A wide variability in the performance of serological tests is a common feature of several published evaluation results (Steingart et al., 2007). Differences in the types, the number and the chemical nature of the antigens used in TB serological tests may be partly responsible for this variability in performance (Steingart et al., 2009). Furthermore, the local epidemiological factors such as the prevalence of HIV, exposure to environmental mycobacteria, vaccination with BCG and the proportion of disease caused by nontuberculosis mycobacteria (NTM) may affect the sensitivity and specificity of a serological antibody test in a particular epidemiological setting (Sada et al., 1992, Yañez et al., 1986). The heterotypic nature of immune responses to antigens of *M. tuberculosis* in different individuals and the different profile of antigenic proteins of *M. tuberculosis* recognized by antibodies at different stages of infection and disease progression are other factors that complicate serological antibody detection in the diagnosis of tuberculosis (Khan et al, 2008, Davidow et al, 2005, Lyashchenko et al., 1998, Botamley, 1995).

The performance of DiaSpot TB Rapid test in this setting was not as good as sputum smear microscopy. However, given the simplicity, the speed and relatively low cost of this test, it may find a use in combination with smear microscopy as a screening test to reduce the number of sputum samples to be examined. In such a use, it may lessen the inconveniences of patients and reduce the laboratory workload.

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