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### ASSESSMENT OF THE DIAGNOSTIC POTENTIAL OF CLINOTECH TB SCREEN TEST FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN NIGERIA

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#### ABSTRACT

The Clinotech TB Screen test, a 3<sup>rd</sup> generation multi-antigen rapid chromatographic immunoassay for detection of IgG antibodies in serum against recombinant protein antigens 38kDa, 16kDa and 6kDa, was assessed for its diagnostic potential for diagnosis of active pulmonary TB in routine TB control programme in Abia State, Nigeria. The overall sensitivity and specificity of Clinotech TB Screen test were 24.1% and 87.8% (95% Confidence intervals [CI]: 14.7-33.5% and 80.6-95.0%) respectively. The positive and Negative Predictive Values (PPV and NPV) were 79.2% and 37.5% respectively. The performance of the test was inferior to that of the sputum smear microscopy which had a sensitivity of 50.0% (95% CI, 39.0%-61.0%) and specificity of 92.3(95% CI: 86.4-98.2%). In 37 culture positive smear positive PTB cases, Clinotech TB Screen test was positive in 18(48.65%). The rapid test showed a very low degree of sensitivity in smear -negative culture positive PTB cases; detecting just one (2.38%) out of 42 cases. These results indicate that the diagnostic value of Clinotech TB Screen test for routine diagnosis of PTB in this setting is limited.

**Key words:** Tuberculosis, serological tests, immunochromatographic tests, rapid TB tests

#### INTRODUCTION

Diagnosis is a key element in the global plan to control TB (1). Rapid and accurate diagnosis is critical to effective TB care and interruption of transmission (2). However, lack of simple, rapid and accurate diagnostic tests remains one of the greatest constraints for effective control of TB, especially in developing countries where the highest burdens of TB are found and facilities for performing traditional laboratory diagnosis are inadequate or not available (3). Furthermore, the existing conventional diagnostic methods such as sputum smear microscopy (SSM), culture and chest radiography have technical and operational characteristics that limit the quality and the scope of their applications in developing countries (4).

Rapid Diagnostic tests (RDTs) employing immunological principles to detect antibodies have recently been developed. These tests are attractive because they are easy to use, rapid, and relatively inexpensive and without the need for sophisticated laboratory infrastructure (5). They hold out a great promise of providing the developing countries with an invaluable way to quickly and cheaply diagnose infectious diseases (6).

Many antigens of *Mycobacterium tuberculosis* have been isolated, purified and tested for their potential for development of rapid serological tests (7). Some of these antigens have been employed in developing commercial serological TB tests (8). Clinotech TB Screen from Clinotech Diagnostics and Pharmaceuticals, Inc, Canada, is one of such tests being distributed in several developing countries including Nigeria. Evaluation of several rapid TB serological tests showed that the tests have variable performance in different epidemiological settings (5). We conducted the present study to assess the diagnostic performance characteristics of Clinotech TB Screen for diagnosis of pulmonary tuberculosis in Abia State, Nigeria.

#### MATERIALS AND METHODS

##### Study Participants.

The study participants were recruited from TB suspects, defined as patients with cough of at least 3 weeks' duration referred to the Leprosy and Tuberculosis Referral Hospital, Uzuakoli, Bende Local Government Area (LGA) and Aba South LGA Health Office, Aba, two major directly observed treatment short course (DOTS) centres within the Abia State TB Control Programme, to undergo sputum smear

microscopy investigation for pulmonary tuberculosis (PTB) between November 2008 and February 2010. Eligible study participants were TB suspects 15 years and above who were not on antituberculosis treatment at the time of recruitment. The study included 150 TB suspects who met the inclusion criteria and were prospectively enrolled as study participants. Informed consent for participation was obtained from the study participants. The study protocol was approved by the Ethical Committee of the Federal Medical Centre (FMC), Umuahia, Abia State.

Three sputum samples, one spot sample on the first day of patient visit, an early morning sample brought from home the next day, and the second spot sample collected when the patient brought the early morning specimen, were obtained from each study participant. Venous blood samples were also collected from the TB suspects for serological TB tests. The blood samples were collected into anticoagulant free plastic tubes from which the sera were later separated.

#### Sputum smear microscopy and sputum culture

For sputum smear microscopy, direct sputum smears were made from the sputum samples and stained by the Ziehl-Neelsen staining technique for acid-fast bacilli (AFB). For culture, the sputum samples were decontaminated using modified Petroff's alkali method and concentrated by centrifugation at 3,000rpm for 30 minutes. The processed sputum samples were cultured on Lowenstein-Jensen (LJ) medium and incubated at 37°C and examined for growth daily for the first 7 days and twice weekly thereafter up to 8<sup>th</sup> week until a definite result was obtained. The slants that did not showed any growth after 8 weeks of incubation were recorded as culture negative.

#### Clinotech TB Screen test

The Clinotech TB Screen test was manufactured by Clinotech Diagnostics and Pharmaceuticals, Inc, Canada and distributed in Nigeria by Clinotech Nigeria Limited, Lagos. The Clinotech TB Screen test is a 3<sup>rd</sup> generation chromatographic immunoassay (CIA) using direct binding double sandwich antibody (Ab-Ag-Ab) principle for direct qualitative detection of TB antibody in human serum or plasma. The test is a multi-antigen test containing recombinant *M. tuberculosis* antigens 38kDa, 16kDa and 6kDa conjugated to colloidal gold particles and immobilized on the test membrane.

The test was done according to the manufacturer's instructions contained in the package insert. The test device was removed from the pouch and placed on a clean, dry level surface. Using the plastic pipette

provided in the test pouch, serum was drawn up to the 100µl mark on the pipette and applied into the sample application well. The test result was read at 15 minutes after sample application and interpreted according to the manufacturer's instructions.

## RESULTS

Clinotech TB Screen test was compared with sputum smear microscopy in 150 TB suspects. The sputum samples of 52(37.14%) of the patients were positive for AFB. Clinotech TB Screen test was positive in 26 (50.00%) of the 52 smear positive cases and 6 (6.12%) of the 98 smear negative cases. In total the Clinotech TB Screen was positive for anti-TB antibodies in 32 (22.86%) of the total study participants. The ability of the test to detect the smear-positive cases according to AFB grading is shown in Table 1. The ability of the Clinotech TB Screen test to identify the culture positive PTB cases is shown in Table 2. In 37 culture and smear positive PTB cases, the test was positive in 18 (48.65%) and in 1 (2.38%) of 42 culture-positive but smear-negative PTB cases.

TABLE 1: PERFORMANCE OF CLINOTECH TB SCREEN TEST IN SPUTUM SMEAR POSITIVE PTB PATIENTS IN ABIA STATE

AFB Grade	No. (%) Positive by SSM	No. (%) Positive by Clinotech TB Screen test
Scanty (1-9) <sup>a</sup>	6/52 (11.54)	1/6 (16.67)
1+ (10-99) <sup>b</sup>	14/52 (26.92)	9/14 (64.29)
2+ (1-10) <sup>c</sup>	16/52 (30.77)	9/16 (56.25)
3+ (>10) <sup>d</sup>	16/52 (230.77)	7/16 (43.75)
<b>TOTAL</b>	<b>52 (100)</b>	<b>26/52 (50.00)</b>

<sup>a</sup> 1-9 AFB/100HPF (high power field)

<sup>b</sup> 10-99 AFB/100HPF

<sup>c</sup> 1-10 AFB/HPF

<sup>d</sup> >10AFB/HPF

SSM- Sputum smear microscopy

The overall performance of the test against a reference standard of sputum culture is presented in Table 3. The sensitivity and specificity were 24.1% and 87.8% respectively. The positive and negative predictive

values were 79.2% and 37.5% respectively. The combination of the results of Clinotech TB Screen test with those of smear microscopy resulted in the

improvement of the sensitivity from 24.1% to 51.4% but with a corresponding decrease of specificity from 87.8% to 80.9%.

TABLE 2: DETECTION OF CULTURE POSITIVE PTB CASES BY CLINOTECH TB SCREEN TEST

Diagnostic category	No. of cases	No. Positive (%)	No. Negative (%)	No. (%) with low signal result (Faint)
Culture positive, smear positive (Cx +ve, Sm +ve)	37	18(48.65)	19 (51.35)	1 (5.55)
Culture positive, smear negative (Cx +ve, Sm -ve)	42	1 (2.38)	41 (97.62)	0 (0)
Culture positive, smear positive (*NTM)	8	3 (37.50)	5 (62.25)	0 (0)
Culture positive, smear negative (NTM)	4	2 (50.00)	2 (50.00)	0 (0)

\*NTM - Nontuberculous mycobacteria

TABLE 3. SENSITIVITY, SPECIFICITY, PPV AND NPV OF CLINOTECH TB SCREEN TEST IN REFERENCE TO SPUTUM CULTURE

Diagnostic tests							
Sputum culture							
	Positive	Negative	Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Clinotech TB Screen test							
Positive	19	5	24	24.1 (14.7-33.5) <sup>a</sup>	87.8 (80.6-95.0)	79.2 (70.2-88.2)	37.5 (26.8-48.2)
Negative	60	36	96				
Total	79	41	120				

<sup>a</sup>95% CI- 95% Confidence Interval

PPV- Positive Predictive value

NPV- Negative Predictive Value

## DISCUSSION

We have assessed the diagnostic potential of Clinotech TB Screen test for diagnosis of active pulmonary TB in routine TB control programme in Abia State, Nigeria. The performance of the test was

inferior to that of the sputum smear microscopy. While the sputum smear microscopy had a sensitivity of 50.0% (95% Confidence interval [CI], 39.0%-61.0%), the Clinotech TB Screen test had a sensitivity of 24.1%

(95% CI: 14.7%-33.5%). In 37 culture positive smear positive PTB cases, Clinotech was positive only in 18(48.65%). The rapid test showed a very low degree of sensitivity (2%), detecting just one of 42 cases of smear –negative culture positive PTB cases in this study. The overall sensitivity and specificity of Clinotech TB Screen test were 24.1% and 87.8% (95% CI: 80.6-95.0%) respectively. The positive and Negative Predictive Values (PPV and NPV) were 79.2% and 37.5% respectively. These values indicate that the diagnostic value of Clinotech TB Screen test for routine diagnosis of PTB in this setting is limited. Previous evaluations of serological TB tests in Nigeria showed variable performance of low to moderate sensitivities. The ELISA-based Pathozyme TB EIA kits (Pathozyme-TB Complex Plus, Pathozyme Myco A, Myco M and Myco G) were evaluated in Lagos by Enwuru *et al.* (9). These kits employed 38kDa and 16kDa antigens as in Clinotech TB Screen test. The authors reported sensitivities of 4% for Myco A, 24% for Myco M and 76% for Myco G with specificity of 100% for Myco G in smear and culture positive category. The performance of Clinotech TB Screen test in our study appeared to be lower than that of Myco G which is the kit in that series that was directed at detecting IgG antibodies. Ohanu *et al.* (10) reported the evaluation of the SmartCheck TB immunochromatographic test in a hospital-based study in Enugu, Nigeria. They used sputum smear microscopy as reference standard and compared the test with X-ray. The sensitivity was reported as 89.5% and the specificity was 77.7%. It is difficult to make a direct comparison of our results with this study because we used culture as reference standard and because the identity of mycobacterial antigen used in the SmartCheck TB was not stated in the paper. Our results are similar to those of Kassa-Kelembho *et al.* (11) who reported a sensitivity of 20.6% and specificity of 90.3% for SDHO MTB test in Bangui, Central African Republic.

Poor diagnostic performance of rapid TB tests has been reported in several previous studies. In a study conducted by Pottumarthy *et al.* (12) to compare the

accuracy of seven antibody detection tests for diagnosis of tuberculosis in New Zealand among immigrants from countries with a high prevalence of tuberculosis, the sensitivity ranged from 16% to 57%. The two immunochromatographic tests, ICT Tuberculosis and Rapid Test TB, included in that study had sensitivity of 41% and 25% respectively. The corresponding specificities were 96% and 87% respectively. The results of laboratory –based evaluation of 19 commercially available rapid diagnostic tests for TB sponsored by Special Programme for Research and Training in Tropical Diseases (TDR) between 2005 and 2006 showed that the sensitivity of the tests ranged from 0.97% to 59.7%; specificity ranged from 53% to 98.7% (13). Clinotech TB Screen test was not included in the TDR study because the manufacturer declined to participate in the study. The evaluation of this test is reported for the first time in this study. Anderson *et al.* (14) evaluated three ELISA based TB antibody tests, InBios Active Tb Detect, IBL *M. tuberculosis* and Anda Biologics TB which utilized newer mycobacterial antigens: Mtb81, Mtb8, Mtb48, DPEP (MPT32) in addition to 38-KDa protein, and two additional proprietary antigens. The sensitivity of InBios Active Tb Detect was 83.3%, IBL *M. tuberculosis* had sensitivity of 5.6% and Anda Biologics TB had 83.3% with corresponding specificities of 98.9%, 100% and 72% respectively.

Although we found the Clinotech TB Screen test to be a rapid and easy to use test, the low sensitivity of the test makes it unsuitable for use as a stand alone test for routine diagnosis of TB in this setting.

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