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Evaluation of Commercial Rapid Diagnostic Test Kit for Tuberculosis: Further Evidence Supporting Negative Policy on the Use of Serological Tests for Pulmonary Tuberculosis Diagnosis in Developing Countries

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Authors' contributions

This work was carried out in collaboration between all authors. Author AJ performed statistical analysis, managed literature searches and wrote the first draft. Author KO designed the study and performed laboratory work. Author NE performed the laboratory work. Author SD performed statistical analysis. Author LL co-ordinated the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To know whether one of the commercially available immunochromatographic tuberculosis tests is comparable with the widely available method, direct sputum microscopy.
Design: The study prospectively validated the pulmonary tuberculosis rapid test kit using the reference standard, Lowenstein Jensen culture and compared the outcome with the direct sputum microscopy.
Place and Duration: The study was conducted in Zankli Medical Centre, Abuja, between November 2004 and July 2005.

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Methodology: 340 patients from direct observation therapy clinics located in six different government owned health facilities were referred to our facility. These patients; male (192) and female (148) were between the age of 10 and 64 years old. Three sputa samples were collected over two consecutive days and direct microscopy and culture were performed on these samples. Also, 4ml of blood were collected from the same patients for antibody detection using immunochromatographic technique.
Results: The evaluated rapid diagnostic kit when compared with the reference standard has a sensitivity of 59.3% and 81.1% specificity. Sensitivity and specificity of direct microscopy, when compared with the rapid test is statistically significant (*P*=0.001); indicating diagnostic accuracy of the conventional method of pulmonary tuberculosis testing over the immunochromatographic test.
Conclusions: The conventional test indicated high performance in this report and it is suggestive of the relevance and diagnostic accuracy of the widely available method in the diagnosis of pulmonary tuberculosis in developing countries. This assertion is also, supported by the 2008 WHO/TDR report on evaluation of nineteen tuberculosis rapid diagnostic kits.

Keywords: Tuberculosis; developing countries; rapid diagnostic kit.

1. INTRODUCTION

Pulmonary tuberculosis (TB) is a preventable and curable infectious disease, unfortunately it is still regarded as one of the leading causes of mortality and morbidity and this impact is felt mostly in twenty two high-burden countries [1]. Poor diagnosis has been identified as one of the reasons for the continual spread of *Mycobacterium tuberculosis* in the developing countries. This concern has led to invention and evaluation of different approaches to TB laboratory diagnosis by different investigators and their findings have been systematically reviewed and published. These identified approaches considered suitable for developing countries and reviewed include bleach microscopy [2] and serological tests [3]. Although, all these evaluated diagnostic tests have limitations like inconsistent diagnostic accuracy, including the most available direct smear microscopy test per sensitivity in developing countries [3] but only the immunochromatography serological test can be considered as a point of care test which is appropriate for a quick diagnosis and subsequently offers the patient's treatment and care, without going to the laboratory for collection of samples in some settings. Recently, another method called Xpert MTB/RIF was introduced purposely for TB high-burden countries [4]. This technology is based on nucleic acid amplification and only one sputum sample is required. Though this technology is considered simple and rapid but its relevance may not be fully gained as a result of dilapidated or lack of basic amenities/infrastructure required in a medical laboratory in most developing countries. So, for now it is important we identify another approach to improve on the diagnosis of pulmonary TB in a low resource setting like ours.

Serological testing can be dated back to 1898 [5], when patient's serum was agglutinated with *M. tuberculosis*. Since then, there have been different immune-based tests developed for the detection of antibodies to pulmonary tuberculosis [6]. So, this study evaluated one of the point of care TB kits that is readily available in the market, particularly in our setting. This TB rapid diagnostic test (RDT) like HIV and Hepatitis B screening test kits is rapid, cheaper and simple to use.

This study prospectively assessed the diagnostic accuracy of one of the tuberculosis RDTs locally available in the market. The choice for the assessed RDT was based on its simplicity which was determined by the technicians that work in the health facilities where the suspected pulmonary tuberculosis patients were recruited. Like most immunochromatographic tests, this point of care device is a direct binding, double sandwich antigen immunochromatographic test for the detection of antibodies to TB present in human serum or plasma. The device has recombinant TB antigens immobilized on the membrane. According to the manufacturer (Clinotech Diagnostics and Pharmaceuticals Inc, Canada), anti-TB antibodies in serum or plasma binds colloidal gold particle conjugated antigens which form sandwich complexes. The complexes migrate through the membrane which has been pre-coated with recombinant TB antigens on the test line and anti-TB antibodies on the control line.

This study compared the selected RDT with the direct smear microscopy and egg based medium (Lowenstein Jensen, LJ) culture. Sensitivity, specificity, positive and negative predictive values of the TB RDT and direct microscopy when compared with LJ culture as the reference standard, were calculated.

2. MATERIALS AND METHODS

2.1 Settings and Patient Recruitment

340 referred patients from DOTS clinics located in six different government owned health facilities spread across the Federal Capital Territory (FCT), Abuja in Nigeria. These facilities are Maitama District Hospital, Asokoro District Hospital, Wuse General Hospital, Gwagwalada Specialist Hospital, Kubwa General Hospital and Gwarimpa General Hospital. These hospitals are managed through the government agency, Hospital Management Board. The patients were referred to our facility, Zankli Medical Centre. These participants; male (192) and female (148) that were between the age of 10 and 64 years old were prospectively enrolled into the study between November 2004 and July 2005. A verbal informed consent was obtained from these participants and ethical approval was granted by the ethical committee of FCT Hospital Management Board and Zankli Medical Centre. The participants referred from the six sites were assessed for suspected pulmonary tuberculosis. Participants that did not submit three specimens over two day's period and participants receiving anti-tuberculosis treatment were excluded from the study.

2.2 Specimen Collection for Direct Microscopy and LJ Culture

All the 340 participants submitted three sputa specimens over two consecutive days. Thus, a total of 1,020 specimens were collected. The first specimen was collected at first visit to the referred site while the second was collected at patient's home based on the instructions given (early morning prior to brushing) and the third specimen, when the patient brought the morning specimen. Instructions were given on how to produce an appropriate specimen for diagnosis of pulmonary TB. The two spot specimens were produced by patients in an open and free ventilated area of the facility. We performed direct smear on all the specimens collected and randomly selected one specimen for culture. All diagnostic tests gave conclusive results.

2.3 Direct Microscopy

1 by 2 cm smears were made from the purulent part of the sputum, air-dried and heat fixed on a hot plate at 85ºC for 2-3 minutes and stained with ZN method (1% filtered carbol-fuchsin and 0.1% malachite green or methylene blue).

2.4 Microscopic Examination and Interpretation

The smears were read using oil immersion lens (x100) of ordinary light microscope by experienced microscopist who were blinded to the rapid TB test and LJ culture. Positive and negative smears were defined accordingly [7]. A patient is reported smear positive for tuberculosis if at least 1 – 9 AFB is seen in 100 high power fields. For the purpose of this study, we only considered a definitive case detection and not necessarily the grading. Hence, we reported on number of patients diagnosed with active *M. tuberculosis* infection.

2.5 Sputum Decontamination (Modified Petroff Method), Culture and Isolation of *M. tuberculosis*

Sputum for LJ culture technique was randomly selected from three specimens of every participant. To 5 ml of sputum was added an equal volume of 4% sodium hydroxide in a 50ml screw-cap tube. This was capped tightly and shaken to digest the sputum; thereafter allowed to stand at room temperature for 15 minutes with occasional shaking. The mixture was centrifuged at 3,000 X g for 15 minutes. The supernatant was carefully decanted and the deposit was re-suspended with 15ml of sterile normal saline and re-centrifuged again at the same rate. The supernatant was removed and the tube sediment of the second centrifugation was inoculated on LJ agar slope and incubated at 37±2ºC and observed daily for the first three days for possible contamination and thereafter regularly examined at weekly interval for 6 - 10 weeks for the isolation of *M. tuberculosis*. Positive and negative growth controls were always included using wild strains of *M. tuberculosis* complex and sterile distilled water respectively. The isolates were identified by the nitrate reductase test, catalase heat-labile test and ZN smear method.

2.6 Blood Specimen Collection and Analysis for Anti-Tuberculosis Antibodies

Following inclusion into the study, 4ml of blood were collected into plain vacutainers without anticoagulants. The specimens were centrifuged for 15 minutes at 2,000 X g and the serum separated into cryovials and stored at -20^{o}C for serological analysis.

2.7 Serological Assays

The test device removed from the pouch and stored specimens were brought to room temperature (18ºC – 30ºC) prior to testing. All specimens were tested with TB RDT. All analyses were performed on specimens obtained from one blood draw per participant. 10µl of serum was applied into the specimen well of the test device and allowed to migrate over the membrane for 15 minutes before interpreting the results. The readings were carried out at daylight. A specimen was considered positive for *M. tuberculosis* if a test line and a control line was observed, while a negative result indicated only control line. The detection of test line without the control line was considered invalid. The technician was blinded to direct smear and LJ culture results and a senior technician reviewed results in case of doubt.

2.8 Statistical Analysis

Sensitivity, specificity, negative and positive predictive values were calculated using standard definitions [8]. Statistical analysis was performed with Stata SE software version 11 (STATA Corp LP, College station TX, USA). A p-value of <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

340 participants recruited during the 8 months period study profile, is shown in the Fig. 1.. When compared with the reference standard, TB RDT has a sensitivity and specificity of 59.3% and 81.1% respectively, and a negative predictive value of 91.3% (Table 1) indicating the proportion of AFB negative participants were actually not infected with *M. tuberculosis* when tested with TB RDT. Sensitivity and specificity of direct microscopy, when compared with TB RDT is statistically significant (*P*=0.001), indicating diagnostic accuracy of the conventional method of pulmonary tuberculosis testing over the immunochromatographic test.

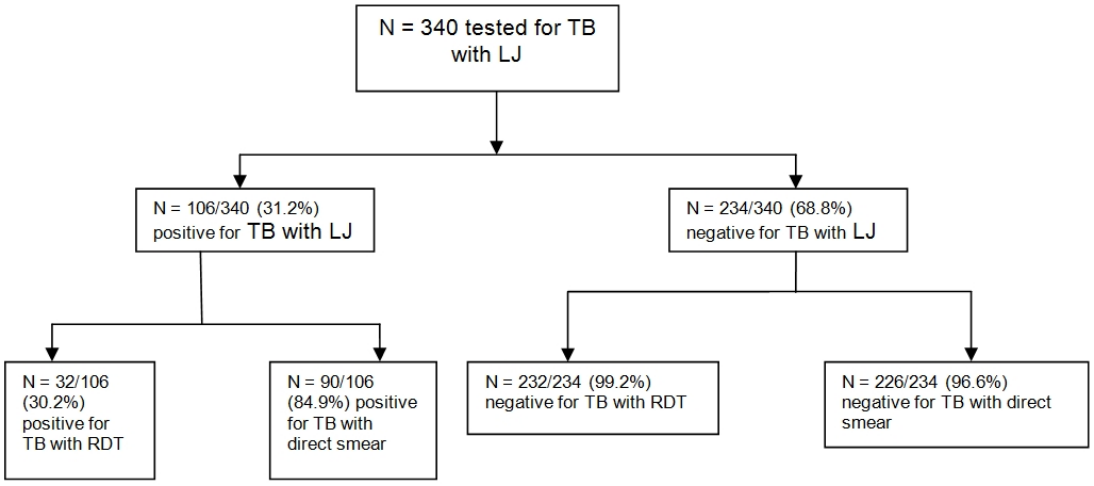

Fig. 1. Enrolment and outcome: Study profile of the evaluation of TB RDT and direct smear against the reference standard

Table 1. Diagnostic tests characteristics of TB RDT and direct smear determined when compared with the reference standard. PPV – positive predictive value, NPV – negative predictive value, CI – confidence interval

	Direct smear		TB RDT		Differences	
Diagnostic accuracy	**AFB outcome n/N**	**% (95%CI)**	**Antibodies outcome n/N**	**% (95%CI)**	**% (95%CI)**	***P* value**
Sensitivity	90/106	84.9 (78.1-91.7)	32/54	59.3 (46.2-72.4)	25.6 (10.9-40.4)	<0.001
Specificity	226/234	96.6 (94.3-98.9)	232/286	81.1(76.6-85.7)	15.5 (10.4-20.6)	<0.001
PPV	90/98	91.8 (86.4-97.3)	32/86	37.2(27.0-47.4)	54.6 (43.1-66.2)	<0.001
NPV	226/242	93.4 m(90.3-96.5)	232/254	91.3(87.9-94.8)	2.0 (-2.6-6.7)	0.391

There exist different formats of serodiagnosis of TB in the market of developing countries and these formats include the immunochromatography, enzyme-linked immunosorbent assay, and agglutination. However, the widely available and used format is the immunochromatography because it is simple, quick and cheaper and appropriate for peripheral laboratories and private laboratories in developing countries. There are different mycobacterial antigens used in the detection of host response to TB and they include 38 kDA, 34 kDA, 16 kDA, LAM, tuberculophosphatide, and Antigen 60 [3]. Although, we had no information on the antigen used for the production of the RDT utilized in this study because of the proprietary nature associated with most diagnostic kits found in the market but most TB RDT uses 38 kDA antigen. This is because 38 kDA has been described as an extracellular lipoprotein involved in phosphate metabolism which is specific for tuberculosis complex (*M. tuberculosis, M. bovis, M. africanum, M. microti*) and has more diagnostic significance when compared with other antigens [9,6].

This study reported a sensitivity and specificity of 59.3% and 81.1% respectively for the TB RDT when compared with the reference standard. Previous reports based on immunochromatography format reported the following sensitivity and specificity respectively; 87% and 82% [10], 40% and 100% [6], 64% and 85% [11]. The sensitivity reported in this study compares favourably with all the published reports, except the one reported by McConkey et al. This indicates a high variability of TB RDT sensitivity available in the market. Our assumption is also supported by a systematic review by Steingart KR et al., 2007 and the World Health Organization report on evaluation of nineteen TB RDTs available in different markets from developing countries [12]. Briefly, WHO/TDR evaluation reported a sensitivity range of 59.7% to 97.0% and 53.0% to 98.7% for specificity.

One of the strengths of our report is the high number of study participants recruited when compared with other published reports [10,6,11] but there were limitations like lack of a control group of apparently healthy population when compared with the published studies. Also, our study did not evaluate the HIV status of the participants unlike the study conducted by Perkins et al. However, when compared with direct smear microscopy, the conventional test indicated high performance in this report. This is suggestive of the relevance and diagnostic accuracy of the widely available method in the diagnosis of TB in developing countries. Again, this assertion is supported by the WHO/TDR report [12].

According to the WHO/TDR report [12], the manufacturer of the kit used in this study declined to subject the manufactured kit for evaluation and when it was enquired from the market (as at the time of writing this report), it seems the kit has been withdrawn from our market.

Reports have implicated the abuse of TB serological tests in high burden countries and its negative impact on the health and economic burden on the patients and health systems [1,13]. Lack of regulation in most developing countries has been identified as one of the reasons; the manufacturers of serological kits in western countries keep exporting these products that failed their regulatory agencies evaluations [1].

4. CONCLUSION

The rapid diagnostic immunochromatography kit is not proper for routine diagnosis of TB because of low sensitivity which implies large percentage of false negative results. Our report supports the evidence as established by WHO/TDR evaluation and the systematic review against the use of rapid diagnostic kits for tuberculosis screening in developing

countries. Our study supports WHO issuing a negative policy against TB RDT [14] use in these countries. However, this policy could only be effective if government agencies implement it by educating the clinicians, laboratorians and the public, and monitors its importation by distributors and collaborating with regulatory bodies in countries where the kits are manufactured to prevent its exportation.

CONSENT

A verbal informed consent was obtained from the study participants.

ETHICAL APPROVAL

All authors hereby declare that the study have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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