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COMPARATIVE ASSESSMENT OF URINE LIPOARABINOMANNAN ASSAY AND ZIEHL NEELSEN SPUTUM SMEAR MICROSCOPY AS TUBERCULOSIS DIAGNOSTIC TOOLS IN NNEWI, NIGERIA

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Abstract

Introduction. Tuberculosis (TB) remains a major public health issue worldwide, with about 8.0 million new cases and 2.0 million deaths yearly, the burden being more in the developing countries partly due to missed or improper diagnosis. Most available methods for TB diagnosis have one shortcoming or the other. The current detection rate of TB is far below set target, making it imperative for diagnostic tests that are rapid and accurate.
Aim: To assess the accuracy of urinary lipoarabinomannan (LAM) antigen and ZN microscopy assays in detecting MTB using Gene Xpert MTB/RIF as the gold standard.
Methodology: A total of 200 participants, 109 males and 91 females, accessing care at the TB (DOTS) Center of Nnamdi Azikiwe University Teaching Hospital, Nnewi were tested for TB using urinary LAM ELISA, ZN sputum microscopy and Xpert MTB/RIF. All the participants were recruited sequentially as they presented at the DOTS clinic. This is an experimental cross sectional study and all the data obtained were analysed using SPSS (statistical package for social sciences), version 20.
Results: ZN microscopy with a sensitivity of 80.0%, specificity of 91.1%, positive predictive value of 90.0%, negative predictive value of 82.2%, and diagnostic accuracy of 85.6% was found to be comparatively superior to LAM ELISA which had sensitivity of 13.3 %, specificity of 71.1% , positive predictive value of 31.6%, negative predictive value of 45.1 and diagnostic accuracy of 42.2%. Positivity rate with LAM ELISA was higher in HIV-positive patients (30.8 %) than in the HIV negative patients (20.0%).

Conclusion: LAM antigen assay does not appear to be very useful as a stand-alone test for TB due to its variable sensitivity and specificity compared to the gold standard in TB diagnosis which is smear microscopy. Smear microscopy should be optimized for enhanced performance in view of its superiority over LAM ELISA as noted in this study.

Key words: *Tuberculosis, Gene Xpert MTB RIF Assay, ZN Staining, LAM ELISA Assay*

Introduction

Tuberculosis (TB) is an air-borne infectious disease caused by various strains of Mycobacteria that usually attack the lungs. TB remains a major public health issue worldwide, with an estimated 8.0 million new cases and 2.0 million deaths annually. The burden is particularly pervasive in the developing regions of Sub- Sahara Africa and South East Asia, both of which have the countries with the world's highest TB burden, accounting for 80% of global TB cases. This situation is partly attributed to delayed, missed or improper diagnosis in these resource-limited countries[1]. Undiagnosed and misdiagnosed TB drives the TB epidemic by creating and maintaining a continuous pool of transmission of the TB organism. There is postponement of initiation of treatment, which potentially leads to increased morbidity and mortality[2].

Rapid and accurate laboratory diagnosis is critical to effective TB treatment[3,4]. The Mantoux tuberculin skin test is only a screening test that measures delayed-type hypersensitivity response to purified protein derivative. Its results may be interfered by Bacilli Calmette- Guerin (BCG) vaccination. It is not confirmatory and a negative result does not rule out TB disease, as may be seen in those with HIV, sarcoidosis and malnutrition. Also, the administration and reading of the TST require a certain amount of expertise that, when lacking, may result in erroneous interpretations[5]. Gamma interferon release assays provide an alternative for the diagnosis of latent TB infection. They show higher sensitivity and specificity, better correlation with exposure to the TB organism, lower cross-reactivity with BCG, hence fewer false-positives compared to Mantoux test. However, there is not yet adequate evidence for the accuracy of these tests for specific populations, including HIV-infected or other immunocompromised patients and children[3,6].

The old Ziehl-Neelsen sputum smear microscopy method remains the method of choice in most resource poor countries because of its relative cost effectiveness in terms of equipment and reagents. However, the method has technical and operational characteristics that limit the quality and scope of its applications. It is tedious and time consuming, thus limiting the number of slides that can be examined per day. It also has the problem of comparative low sensitivity. It detects fewer than 60% of all new TB infections and as few as 20-35% of HIV/TB infections[7]. In Nigeria there is little or no facility at the primary health care centers for reliable diagnosis of TB. Skilled and trained manpower, infrastructure and electricity supply are in short supply or completely non-existent making it difficult to diagnose TB. The method also requires repeated visits to the hospital for sample submission and result collection. Patients drop out in the diagnostic process is common with the result that many TB cases remain undetected and untreated[8]. Use of culture is the gold standard. It allows for

proper identification of the isolated organism and drug sensitivity testing. However, this is not feasible in resource limited settings due to its cost, technicality and prolonged turnaround time[9]. There have been recent impressive advances in the field of TB diagnosis. Several automated molecular techniques (including the Gene Xpert MTB/Rif assay and Line Probe assay) with reduced turn over time have been developed but their high costs or sophisticated infrastructure requirement makes them unaffordable for large scale routine use[10,11].

Perhaps, as part of the solution to this challenge, rapid diagnostic tests that detect TB antigens or antibodies have been developed. These tests are quite attractive because they are easy to use, rapid and relatively inexpensive and without the need for sophisticated laboratory infrastructure. Some formats can be performed in the fields or at the point of care by non-technical staff. So many of these[rapid test kits] are commercially available, but most are not endorsed by any international body[12]. Yet, they are packaged, offered for sale and are widely used in many TB high burden countries[13]. There are contradictory reports in support and against the use of some of these kits, based on their highly variable sensitivity (0- 100%) and specificity (31-100%)[14].

There has been an addition to the list of these assay kits, the urinary TB lipoarabinomannan (LAM) antigen test, which detects the presence of LAM antigen in urine of TB patients. The test is easy to perform and very convenient as urine is easier to collect, and may be less variable in quality and safer to handle. Urine is a particularly more attractive specimen in children who do not have forceful expulsion of sputum. The collection procedure is not invasive. Again, there is no repeated visits to the hospital for sample submission and result collection. The amount of LAM in the urine is said to reflect the bacterial load, hence the assay permits a semi-quantitative assessment of the infectious status[15]. The assay is said to be useful in people living with HIV and children who are disproportionately affected by smear-negative and extra pulmonary TB[16].The increasing sensitivity of urine LAM testing with progressive immune suppression (as reflected by falling CD4 cell counts) is a major distinguishing feature from other TB diagnostics such as sputum smear microscopy which loses sensitivity with worsening immune suppression[17]. As total Mycobacterial burden increases with progressive immune suppression, there is a concomitant increase in the urinary LAM excretion[18].

The Directly Observed Treatment Short course (DOTS) strategy has recorded significant improvement in TB detection in Nigeria. However, the set target for the TB detection rate (at least70%) has not been achieved. The current detection rate of 16% [19] is far below the target and constitutes an impediment in the fight to stop TB. There is the need for early case detection with diagnostic tests that are rapid and accurate if the set TB control targets must be achieved. Most conventional methods for TB diagnosis have one shortcoming or the other as aforementioned. Rapid TB diagnostics such as the urinary TB LAM antigen test has the potentials to close the gap between this current detection rate and target detection rate. The test has many appealing properties for use in resource-limited settings, including cost effectiveness and speed. The lateral flow urine LAM assay (the routine diagnostic) version takes about 25minutes[16,20,21].

However, even as promising as the assay may appear, it is still very important that the

kit be evaluated before it is endorsed for use in Nigeria. This is because evaluation of several rapid TB serological tests has shown that these tests have variable performances in different epidemiological settings[22]. This study was aimed to compare the sensitivity, specificity and diagnostic accuracy of ZN microscopy and urinary LAM Ag in detecting *MTB* using Gene Xpert MTB/RIF as the gold standard.

Methodology

A total of 200 pulmonary TB suspects comprising 109 males and 91 females, of mean age of 29.7 years, 65 of whom were HIV positive and 135 HIV negative and with cough ≥ 2 weeks accessing care at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were used for this study. Patients already enrolled in TB treatment prior to commencement of this study were excluded. Written informed consent was obtained from each of the subjects. Ethical clearance was obtained from the Ethics Committee of NAUTH. Each patient submitted two samples of sputum, one on the spot on first day and the other as early morning sample the second day, according to the national algorithm[5]. Each sample was collected in sterile, wide- mouthed, transparent, leak-proof sputum cups. Each patient also collected early morning urine sample into sterile universal container for detection of urinary TB LAM antigen. Urine samples were frozen- stored as soon as received in the laboratory.

Smear measuring 1cm × 2cm was made from each sputum sample by spreading the sputum in an oval shape, allowed to air dry, heat-fixed and stained by the hot Ziehl Neelsen staining procedure as adopted by National Tuberculosis Programme[5]. Positive and negative control slides were included in each batch of staining. Each stained smear was examined under ×100 oil objective lens and scored according to the WHO/IUATLD system (1980)[23].

Each of the early morning sputum sample for Xpert assay was diluted 2:1(v/v) with the sample reagent, mixed by shaking vigorously 10-20 times and incubated at room temperature for 15 minutes to achieve sample liquefaction and inactivation of *Mycobacteria tuberculosis.* Two (2.0) ml of the diluted sputum was transferred into the Xpert MTB/RIF cartridge, scanned with bar-code scanner to determine its content integrity/expiry date. The cartridge with diluted sample was placed into the module for real time polymerase chain reaction. Results were recorded as: MTB not detected (Negative), MTB detected/RIF resistance not detected (Positive), MTB detected/RIF resistance detected (MDRTB), accordingly at the end of the processing[24].

The urinary LAM ELISA assay procedures were conducted according to the kit producer's instructions. The optical density was measured at 450 nm using a microtiter plate reader within 15 minutes to obtain the LAM concentration. The average concentration for each sample was compared to the cut-off value of 0.98mg/100ml to determine positivity or negativity[25].

The statistical analysis of results was done using SPSS (statistical package for social sciences), version 20, to carry out chi square tests and MedCal software to compare sensitivity and specificity as well as the positive predictive values (PPV), negative predictive values (NPV), likelihood ratios and diagnostic accuracy of ZN AFB microscopy and LAM ELISA methods with Gene Xpert as the gold standard. P value was calculated at 95% confidence level and P values less than 0.05were considered statistically significant.

Results

Ninety (45%) of the 200 patients were diagnosed TB positive using the Gene Xpert MTB/Rif assay, while ZN microscopy and Urinary LAM ELISA detected TB in 82 and 47 of 200 respectively. ZN microscopy showed a sensitivity of 80.0%, specificity of 91.1%, positive predictive value of 90.0%, negative predictive value of 82.2%, and diagnostic accuracy of 85.6% over urinary LAM ELISA with a sensitivity of 13.3 %, specificity of 71.1% , positive predictive value of 31.6%, negative predictive value of 45.1 and diagnostic accuracy of 42.2% as shown in figure 1.

The association between HIV status of the patients and results of the Gene Xpert MTB/RIF assay is shown in Table 1. Eighteen (18) (27.7%) of the 65 HIV positive TB suspects had HIV-TB co-infection. The association between HIV status of the patients and results of the Ziehl Neelsen AFB microscopy technique is shown in Table 2. The Ziehl Neelsen technique was seen to be more sensitive in HIV negatives (48.9%) than in HIV positives (24.6%) (p - value: 0.001). The association between HIV status of the patients and results of urinary LAM ELISA assay which is shown in Table 3 indicates that the positivity rate with LAM ELISA was higher in the HIV positives (30.8%) than in the HIV negatives (20.0%) (p - value:0.092).

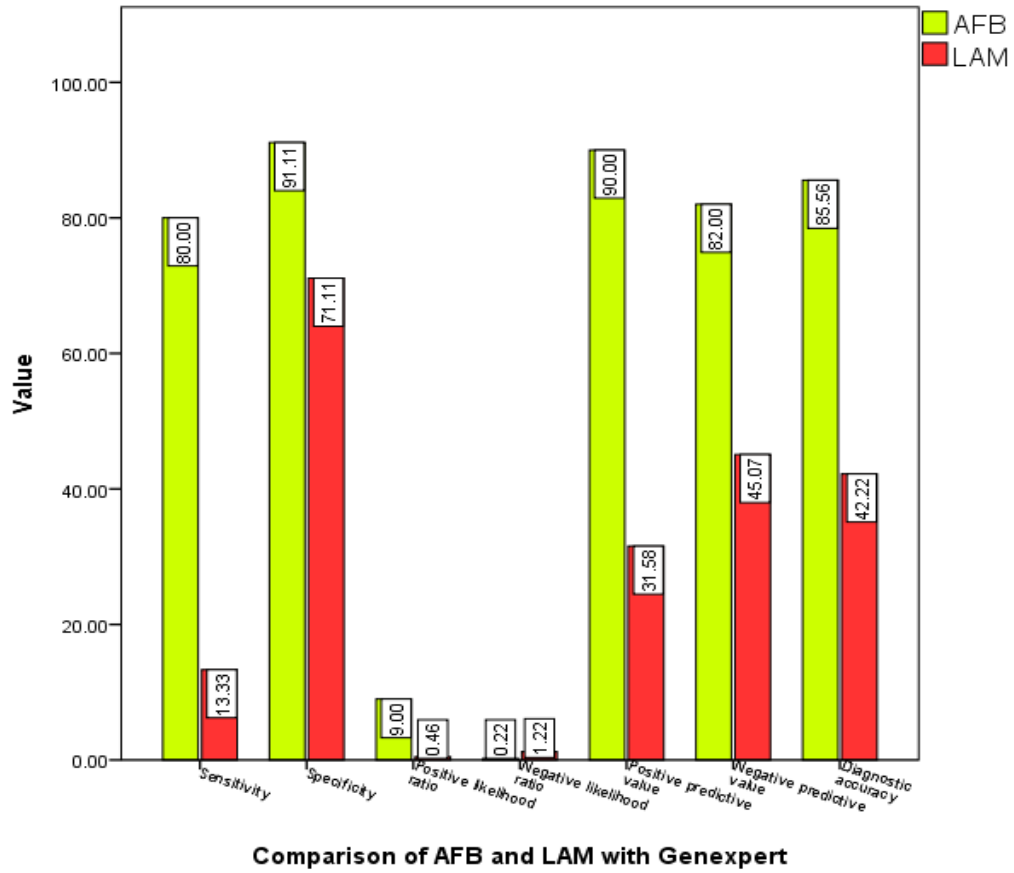

Figure 1 : Comparison between ZN AFB microscopy and LAM ELISA.

Table 1: Association between TB status by Gene Xpert MTB/ RIF and HIV status of patients.

			HIV Status		Total	P-value
			Positive	Negative		
Gene Xpert	Positive	Count (% within HIV Status)	18 (27.7%)	72 (53.3%)	90 (45.0%)	0.001
	Negative	Count (% within HIV Status)	47 (72.3%)	63 (46.7%)	110 (55.0%)	
Total		Count (% within HIV Status)	65 (100.0%)	135 (100.0%)	200 (100.0%)	

Table 2: Association between TB status by AFB and HIV Status of patients.

			HIV Status			P-value
			Positive	Negative	Total	
AFB	Positive	Count(% within HIV status)	16 (24.6%)	66(48.9%)	82 (41.0%)	0.001
	Negative	Count (% within HIV status)	49 (75.4%)	69 (51.1%)	118 (59%)	
Total		Count (% within HIV status)	65 (100%)	135(100%)	200(100%)	

Table 3: Association between TB status by LAM ELISAA and HIV status of patients.

			HIV Status			P-value
			Positive	Negative	Total	
LAM	Positive	Count (% within HIV Status)	20 (30.8%)	27 (20.0%)	47 (23.5%)	0.092
	Negative	Count (% within HIV Status)	45 (69.2%)	108 (80.0%)	153 (76.5%)	
Total		Count (% within HIV Status)	65(100 %)	135 (100%)	200 (100%)	

Discussion

In this study ZN AFB smear microscopy was found to be more sensitive and specific than the LAM antigen test. Smear microscopy has a sensitivity of 80.0% and specificity of 91.1% higher than sensitivity of 13.3% and specificity of 71.1% of LAM ELISA assay. This finding tallies with those of another studies[26-28]. On the other hand some previous studies have evaluated urine LAM antigen test and found out that its sensitivity and specificity were superior to that of sputum smear microscopy[16, 29-35] in Nigeria documented that urine LAM testing was more sensitive (77.8%) than the AFB smear microscopy (33.3%). These variations may be related to operational and technical issues surrounding LAM assay and which impacts its sensitivity and specificity. These include the involved different patient population, technical issues such as the collection of more than one sputum samples or use of sputum concentration or fluorescence microscopy method which are said to increase diagnostic sensitivity of sputum smear microscopy.

Urinary LAM assay has been evaluated globally with widely varying sensitivity and specificity. A meta-analysis of studies using urine LAM assays in patients with microbiologically confirmed TB, reported variable sensitivity ranging from 13% to 93% and specificity ranging from 87% to 99% [36]. The underlying causes of the variable sensitivity and specificity of urine LAM testing are not adequately understood and this has been an obstacle to its wider application because it makes it difficult to compare performance characteristic of LAM across various settings. According to earlier studies, the variability in sensitivity may be attributed to the study design, study populations, hospitalized versus out-patients, HIV status, degree of immunosuppression, sample patient selection, humoral immune response and the proportion of urine LAM derived from either renal or extra-renal TB sources [37]. For instance, sensitivity of LAM assay generally has been found to be higher among hospital inpatients than among outpatients (58-67% versus 17-32%) [16,29,36,38,39]. The patients in this study were not hospitalized or stratified by the severity of their illness. This limitation could have impacted the LAM ELISA sensitivity in this study.

Other factors that can influence the sensitivity of LAM antigen assays include the characteristics and standardization of the

test-capture antibody, variable concentration of the urine sample, unprocessed or centrifuged urine, use of fresh urine versus frozen urine, cut-off grade for positive result and performance of the test by state certified medical laboratory personnel versus trained but not certified hands [37]. LAM ELISA tests that incorporate poly-clonal antibodies for LAM antigen capture are more likely to recognize the multiple antigenic epitopes of LAM compared with those using monoclonal antibodies that are targeted at a single epitope[40] and that was what was used in this present study.

Sample containers, collection, storage and processing could also affect the results of LAM ELISA. Cross reaction of perineal or fecal bacteria can occur in urine contaminated from unsterile container. Microbiological contamination of urine sample in sufficient quantity may affect the specificity of the assay[41,42.] To prevent the possibility of this ugly occurrence in this study urine sample were collected in sterile containers. Concentrating the urine before carrying out LAM ELISA assay is said to increase the sensitivity of the LAM ELISA, but decreases specificity[43].

In this study, LAM ELISA was not performed on fresh but on frozen urine samples and without centrifugation and it is unclear if this could impact the obtained results. However, careful instructions were given to subjects such as recapping the sterile container soon after collection and submitting the sample to the laboratory as soon as possible and the samples were stored frozen until they were processed. Other possible reasons for the variable specificity in LAM ELISA studies include the sensitivity of the gold or reference standard used in the different studies[41]. Some used composite reference standards[44,39] while others used microbiological reference standards which could be culture[21,31] or Gene Xpert MTB/RIF assay[35].

In this study LAM ELISA detected TB in 20 (30.8%) of the 65 HIV patients while Gene Xpert MTB/RIF and ZN microscopy techniques detected TB in 18(27.7%) of the 65 and 16 (24.6%) of the 65 HIV positive patients respectively. Positivity rate with LAM ELISA was higher in HIV-positive patients (30.8 %) than in the HIV negative patients (20.0%), a finding that tallies with those of some previous studies [16,45,46]. It has been observed that urine LAM detection appears to have greater diagnostic accuracy in patients with HIV co-infection with lowered CD4 count than in the HIV negative patients. Studies stratifying urine LAM by CD4 cell count have shown sensitivities of 56- 85% among patients with CD4 <50 cells/⌠l as against a sensitivity less than 25% in HIV-uninfected populations [36]. LAM concentration in urine of HIV negative TB patients is said to be extremely low, in many cases in the range of pico-gram per ml, hence the urine LAM assay which detects LAM in the range of nano gram per ml, may not be sensitive enough for diagnostic use in such unselected TB suspects[15] used in this present study. The stage of HIV infection or severity of immunosuppression of the HIV positive patients in this study was not determined nor was the CD 4 count done [16-18.]

Conclusion

Irrespective of the simplicity and rapidity, the current commercially available generation of LAM antigen assay does not appear to be very useful as an independent stand-alone diagnostic test for TB in view of its variable sensitivity and specificity. And until the Gene Xpert machine becomes adequately available; perhaps, the use of the conventional sputum smear microscopy should be optimized to further enhance its performance considering its superiority over urinary LAM ELISA as observed in this study. Smear microscopy is quite attractive

for public health programs. It is relatively cheap, specific enough and can provide visual evidence of the bacterial burden. Attention should be focused on techniques for optimization of smear microscopy to improve its sensitivity and to reduce diagnostic defaulting by assessing the feasibility of diagnosing PTB with two sputum samples on a single day known as 1-day protocol or front-loading, particularly for patients who are more likely to default. Training/retraining for laboratory scientists in smear microscopy and external quality assessment will be needful.

Recommendation

There is the need for further researches to.

1. Increase the evidence base for the diagnostic accuracy in the appropriate clinical populations by characterization of patients using parameters such as symptom profile, CD4 cell count, blood hemoglobin, body mass index as well as morbidity tools such as Modified Early Warning Score (MEWS). These may help in the interpretation and comparison of study data in future studies.
2. Define the optimum methods of urine collection to minimize the likelihood of urine contamination and optimum storage if analyses are to be done.
3. Incorporate the assay into the national diagnostic algorithm.
4. Standardize the cut-off mark.
5. To optimize the conventional sputum smear microscopy to further enhance its performance
6. Ensure wider distribution of the Gene Xpert Machine to more facilities.
7. To enhance the sensitivity of smear microscopy.

References

1. World Health Organization. Media Centre. Tuberculosis Fact sheet. 2016;3(6) http://www.who.int/mediacenter/factsheets/fs104/en/
2. Small P. M., Pai M. Tuberculosis diagnosis- time for a game change. *New England Journal of Medicine*.2010;363:1070-1071.
3. Pai M., Ramsey A., Obrien R. Evidence-based Tuberculosis Diagnosis. *PLOS medicine.* 2008;5(**7**):156.
4. Raviglione M.C. The new stop TB strategy and the global plan to stop TB, 2006-2015.*Bulletin of the World Health Organization*. 2007;85(**5**):327
5 Federal Ministry of Health (FMOH). Department of Public Health: National Tuberculosis, Leprosy and Buruli Ulcer Management and Control Guidelines (NTBLCP). 2015; 6th edition.
6. Parsons L.M., Somoskoyi A.,Gutierrez C., Lee E.,Paramasivan C.N ., Abimiku A .,Spector S.,Roscigno G., Nkengasong J. Laboratory Diagnosis of Tuberculosis in Resource-Poor Countries: Challenges and Opportunities. *Clinical Microbiology Review.* 2011;24(**2**): 314-350.
7. Steingart K.R., Megan H., Virenne N.G. Sputum processing method to improve the sensitivity of smear microscopy for *tuberculosis*: A Systematic Review. *The Lancet Infect. Dis.*, 2009;6 (**10**): 664-677.
8. Yimer S.M., Holm-Hanse C., Yimadu T., Bjune G. Evaluating an active case-

finding strategy to identify smear positive tuberculosis in rural Ethopia. *International Journal of Tuberculosis and Lung Diseases.* 2009; **13**:1399-1404.

9. Cuevas L. E., Vassin M.A., Al-Sonboli L. N., Bahader J. A multi-country non–inferiority cluster randomize trial of front–loaded smear microscopy for the diagnosis of pulmonary tuberculosis. *PLOS medicine.* 2011;8(**7**):403.
10. Saroj H., Nital P., Bharti M., Sumit G., VipinK., Leela V. Comparison of Ziehl- Neelsen and Auramine staining methods on direct and concentrated smears in clinical specimens. *Indian Journal of Tuberculosis.* 2011; **58:72-**76.
11. Pai M., Kalanti S., Dhehe K. New tools and emerging knowledge for the diagnosis of tuberculom: Part II. Active tuberculosis and drug resistance. *Expert Review of Molecular Diagnostic*. 2006; **6:423-**432.
12. David A., Kananfia G. V., Shi L., Kaviar J., Guo X. Antibody profiles characteristic of mycotuberculosis infection state. *Infection and Immunity*. 2005;73(**10**): 6846-6851.
13. Grenier J., Pinto L., Naiv D., Teingart R. R., Dowdy D., Ramsay A. (2012). Widespread use of serological tests for tuberculosis: data from 22 high burden countries. *European Respiratory Journal.* 2012;**39**: 5
14. Steingart K.R., Flores L.L., Dendakari N., Schiller I., Laai S., Ramsay A., Aperell P.C., Pai M (2011). Commercial serological tests for diagnosis of active pulmonary and extra pulmonary tuberculosis: an update systemic review and meta-analysis. *PLOS Medicine.* 2011;8(**8**):62-71.
15. Hamasur B., Bruchfeld J., van Helden P., Källenius G., Stefan S. A Sensitive Urinary Lipoarabinomannan Test for Tuberculosis. *PLoS One* 2015;10(**4**): 457.
16. Lawn S.D., Kerkhoff A.D., Vogt M., Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. *Lancet Infectious Diseases.* 2012;(**3)**:201-209.
17. Lawn S. D., Edwards D.J., Kranzer K., Vogt M., Bekker L.G., Wood R. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. *AIDS*. 2009;23(**14**):1875–1880.
18. Reid M.J., Shah N.S. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. *Lancet Infectious Diseases*.2009; **9**:173–184.
19. World Bank Group. Tuberculosis case detection rate (%, all forms). 2016
20. Brennan P.J. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis. Tuberculosis* (Edinburgh). 2003;83, 91–97
21. Drain P.K., Lilishia Gounder L., Faieza Sahid F., Mahomed-Yunus S. M. Rapid Urine LAM Testing Improves Diagnosis of Expectorated Smear-Negative Pulmonary Tuberculosis in an HIV-endemic Region. *Scientific Reports* 2016;96(**6**): 1038-1055.
22. Ekundayo E. O., Abbey S. D., Achi O. K. (2012)**.** Assessment Of the

Diagnostic Potential Of Clinotech TB Screen Test for Diagnosis Of Pulmonary Tuberculosis in Nigeria. *African Journal of Clinical And Experimental Microbiology*.2012;13(**1**):56-60.

23. International Union Against Tuberculosis and Lung Diseases (IUATLD)(2000), technical guide: sputum examination for tuberculosis by direct microscopy in low income countries. 2000;5th edition.
24. Cepheid International Gene Xpert Operator's Manual and Xpert MBT/RIF protocol.2013
25. Melsin Medical Company Limited, Kuancheng District, Changchun, China .LAM ELISA kit. 2018
26. Steingart K.R., Henry M., Ng V., Hopewell P.C., Ramsay A., Cunningham J. (2006) Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infectious Diseases.* 2006;6(**9**):570–581.
27. Balcha T. T., Winqvist N., Sturegard E., Skogmar S Reepalu A., Jemal Z. H., Tibesso G., Thomas S. T., Bjorkman P. Detection of lipoarabinomannan in urine for identification of active tuberculosis among HIV-positive adults in Ethiopian health centres, *Tropical Medicine and International Health.* 2014;19(**6**):734–742.
28. Bjerrum S., Kenu E., Margaret L. M., Newman M. J., Kwasi Addo K., Johansen I. S. Diagnostic accuracy of the rapid urine lipoarabinomannan test for pulmonary tuberculosis among HIV-infected adults in Ghana–findings from the DETECT HIV-TB study. *Biological Medical Central Infectious Journal.* 2015;15: 407-408.
29. Peter J.G., Theron G, Van Zyl-Smit R, Haripersad A, Mottay L, Kraus S. Diagnostic accuracy of a urine LAM strip-test for TB detection in HIV-infected hospitalized patients. *European Respiratory Journal.* 2012;40(**5**):1211–1220.
30. Peter J.G., Theron G., Dheda, K. Can point-of-care urine LAM strip testing for tuberculosis add value to clinical decision making in hospitalised HIV-infected persons? *PLoS One*. 2013;8(**2**):e54875
31. Shah M., Ssengooba W., Armstrong D., Nakiyingi L., Holshouser M., Ellner, JJ. Comparative performance of urinary lipoarabinomannan assays and Xpert MTB/RIF in HIV-infected individuals. *Acquired Immune Deficiency Syndrome*. 2014; 28(**9**): 1307–1314.
32. Drain P. K., Losina E., Coleman S. M., Giddy J., Ross D., Katz J. N. Diagnostic accuracy of a point-of-care urine test for tuberculosis screening among newly-diagnosed HIV-infected adults: a prospective, clinic-based study. *BMC Infectious Diseases.*2014;14:110.
33. Nakiyingi L, Moodley V. M., Manabe Y.C.,Nicol M. P., Holshouser M., Armstrong D. T. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. *Journal of Acquired Immune Deficiency Syndr* 2014; 66(3):270-9.
34. Drain P. K., Losina E., Coleman S. M., Giddy J., Ross D., Katz J. N. (2015). Value of urine lipoarabinomannan grade and second test for optimizing clinic-based screening for HIV-associated pulmonary tuberculosis. *Journal of Acquired Immune Deficiency Syndrom* 68(3):274–280.
35 Aneio-Okopi A., Okojokwu JO., Adetunji JJ., Onoja A., Folashade A., Ikwulono G., Ebonyi O. A., Ejeliogu

E., Audu, O. Detection and Diagnostic Accuracy of Rapid Urine Lipoarabinomannan Lateral-Flow Assay in Pulmonary Tuberculosis patients in Nigeria. *Trends Journal of Sciences Research*. 2018;3 (1) 52–59.

36. Minion J, Leung E, Talbot E, Dhedha K, Pai M. Diagnosing tuberculosis with urine lipoarabinomannan systematic review and meta-analysis. *European Respiratory Journal*. 2011; 38:1398–405.

37. Wood R., Racow K., Bekker.LG., Middlekoop K., Vogt M., Kreiswirth B.N., Lawn S.D. Lipoarabinomannan in urine during tuberculosis treatment: association with host and pathogen factors and mycobacteriuria. *BMC Infectious Disease*. 2012;8; **12:47**.

38. Shah M., Variava E., Holmes C.B., Coppin A., Golub J.E., McCallum J., Wong M., Luke B., Martin D.J., Chaisson R.E. Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a High HIV prevalence setting. *Journal of Acquired Immune Deficiency Syndrome* 2009;52:145–151.

39. Zijenah L.N., Kadzirange G., Bandason T., Chipiti M.M., Gwambiwa B., Makoga F., Chungu P., Kaguru, P., Dheda K. Comparative performance characteristics of the urine lipoarabinomannan strip test and sputum smear microscopy in hospitalized HIV-infected patients with suspected tuberculosis in Harare, Zimbabwe. *Biological Medicine of Central Infectious Diseases Journal*. 2016;16:20-21.

40. Lipman N.S., Jackson L.R., Trudel L.J., W-Garcia F. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications and information sources. *Institute of Laboratory Animal Research Journal*. 2005; 46:943–954.

41. Dheda K., Davids V., Lenders L., Roberts T., Meldau R., Ling D., Brunet L., van Zyl R., Peter J., Green C. Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. *PLoS ONE*, 2010;5 :e9848.

42. Pearce E.C., Woodward J.F., Nyandiko W.M., Vreeman R.C., Ayaya S.O. (2012) A systematic review on clinical diagnostic systems used in diagnosis of tuberculosis in children. *AIDS Research Treatment*: 2012;1–11.

43. Savolainen L., Kantele A., Sandboge B., Siren M., Vallealle H., Tuompo R., Pusa L., Erkinjuntti-Pekkanen R., Knuuttila A., Ku C., Chi C.,Vasnkan T., Tuuminen T. Modification of Clearview Tuberculosis (TB) Enzyme-Linked Immunosorbent Assay for TB Patients Not Infected with HIV. *Journal of Clinical and Vaccine Immunology* 2013;20(**9**):1479-1482.

44. Rawizza H.E., Oguche S, Imade G., Agbaji O., Olaleye D., Odaibo G., Osinusi K., Adewole I., Okonkwo P., Kanki P. Screening for Tuberculosis in HIV-Infected Nigerian Children with a Urinary Antigen Assay. *Conference on Retroviruses and Opportunistic infections*, Boston, Massachusetts, United States of America.2014; 810. *https://www.croiconference.org/abstract/screening-tuberculosis-hiv-infected-nigerian-children-urinary-antigen-assay/*