ORIGINAL ARTICLE

ACCURACY ON DIAGNOSTIC PERFORMANCE OFPOINT OF CARE LIODETECT® TB-ST RAPID TEST FOR THEDIAGNOSIS OF PULMONARYTUBERCULOSIS IN NORTHWEST ETHIOPIA

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ABSTRACT

Background: *Tuberculosis (TB) is the leading cause of death due to a single infectious agent. To improve TB case detection and treatment in healthcare-disrupted urban and rural settings, an accurate point-of-care (POC) test that does not rely on sputum quality is urgently needed.*

Objective: *To evaluate the accuracy of performance of a new rapid POC antibody-based detection test for the diagnosis of pulmonary TB.*

Methods*: This prospective cross-sectional study was conducted from June 2018 to July 2019 in presumptive pulmonary TB (PTB) patients in Northwest Ethiopia. Sputum was routinely collected for diagnosis of TB with a Lowenstein Jensen (LJ) culture and an Xpert MTB/RIF test was compared with the LIODetect TB ST rapid point-of-care diagnostic test. Using LJ culture and Xpert MTB/RIF for M.tb detection as our reference standards. SPSS ver. 20 and Medcalc were used to analyze descriptive and diagnostic accuracy tests, respectively.*

Result: *A total of 233 samples were analyzed. The LIODetect TB-ST rapid test showed a sensitivity of 53.0%, specificity of 91.0%, positive predictive values (PPV) of 70.0 %, negative predicted values (NPV) of 83.1 % with overall accuracy of 80.3% when compared to the LJ culture as a reference method. Similarly, the same test showed sensitivity of 69.6%, specificity of 90.6%, PPV of 67.9%, NPV of 91.1%, and over all accuracy of 85.8% when compared to the Xpert MTB/RIF as a reference standard. Further, when exclusively evaluated in people living with HIV (PLWH), the LIODetect TB-ST rapid test diagnostic showed similar performance as described above, with an overall accuracy of 83.7% and 86.1% when compared to LJ culture and the Xpert MTB/RIF test, respectively.*

Conclusion*: The LIODetect TB-ST rapid test performance was optimal against the MTB/RIF test and LJ culture. This POC test also had optimal sensitivity and better specificity in both HIV negative people and People living with human immunodeficiency virus (PLWH). Thus, the LIODetect TB-ST rapid test could be a useful as POC TB screening test in resource-limited urban/ rural areas, where routine TB diagnosis using LJ and/or the Xpert MTB/RIF test can not be performed.*

Keywords: *Mycobacterium tuberculosis, diagnostics, point-of-care, LIO detect TB-ST, Xpert MTB/RIF.*

INTRODUCTION

Tuberculosis (TB) is the second leading infectious disease cause of death due to a single infectious agent after Corona virus Disease 2019 (COVID-19) [REF: WHO COVID and TB report]. Globally, an estimated one fourth of the world population is infected by *Mycobacterium tuberculosis (M.tb).* Despite decades of effective treatment being available, in 2019, 10 million people felt ill and 1.4million died of active TB (1-3). This is further fueled with the emergence of multidrug-resistant (MDR) TB.(4).

TB is still a major health problem in Ethiopia with thousands of cases reported yearly(5). Undiagnosed

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or delayed TB diagnosis and treatment mismanagement drive infection transmission, significantly obstructing TB prevention and control efforts(6). This is currently more noticeable due to the COVID-19 pandemic (7-9), where scarce resources for TB diagnosis as well as front-line TB staff have been diverted for mitigating the COVID-19 pandemic efforts(10-12).

The End TB Strategy goals, which aim to achieve a 90% and 95% reduction in TB incidence and mortality, respectively, by 2035, will not be achieved without new tools to detect TB prevalence in rural areas of low-income nations(13). These include improved point-of-care (POC) diagnostic tests distributed at the most decentralized levels of care where patients first contact the health system, and within the community (13). These tests should be achievable on non-invasive, easily obtainable samples with test results provided at the point-of-care, allowing for rapid initiation of treatment and for complete diagnosis, treatment and care in a single clinical encounter, hence avoiding delay in care and patient loss-to-follow-up (13, 14).

One of the TB control principles is a rapid and accurate diagnosis of TB patients in order to allow prompt initiation of anti-TB drug therapy and to prevent transmission (15).However, currently available TB diagnostic tests depend on sputum samples, which are difficult to get and have low diagnosis sensitivity in immunocompromised patients, disseminated TB patients, and children, delaying their treatment initiation. Approximately 36% of all TB cases and 49% of TB cases among people living with Human immunodeficiency virus (PLWH) are not diagnosed or reported, which may contribute to the increase in TB prevalence, especially in low-income nations. This gap is partly explained by limited methods available to diagnose TB especially for those who are unable to produce sputum and have frequent extrapulmonary and/or paucibacillary disease.

To improve TB case detection and treatment, accurate point-of-care (POC) tests that do not rely on sputum are urgently needed(16, 17). WHO has prioritized non-sputum, biomarker-based, POC tests that can be used to rapidly diagnose pulmonary and extrapulmonary TB including use in children(16). Currently, there are several commercially available urine-based diagnostic tests: the Fuji LAM and the Alere Determine LAM-Ag detection tests(18). Both detect the presence of a major cell wall component of the *M. tuberculosis* complex, the lipoarrabinomannan (LAM), in urine. Studies evaluating Fuji LAM *vs.* the Alere Determine LAM-Ag tests performance in different populations against a microbiological reference standard showed that the Fuji LAM test had a sensitivity of 64.9% and 74.2% with a specificity of 83.8% and 89.3% among children and people living with HIV (PLWH), respectively(19, 20).The Alere Determine LAM-Ag test had a sensitivity of 30.7% and 53.0% with specificity of 87.8% and 95.6% for children and PLWH, respectively(19).

Another POC diagnostic platform is the use of whole blood assay. The LIODetect TB-ST Rapid Test (Lionex diagnostic and therapeutic GmbH, Braunschweig, Germany), detects antibodies against LAM and a series of *M. tuberculosis complex* proteins. Based on the manufacturer reports, from both in house and an external study using only clinical diagnosis as a reference standard, the LIODetect TB-ST test had a sensitivity and specificity of 65.4% and 96.6%,respectively, in children, and a sensitivity and specificity of 72.8%and 94.0% respectively, in PLWH (21).

In order to assess the performance of the LIODetect TB-ST test in a field setting, we evaluated the performance of this test for the diagnosis of pulmonary TB (PTB) presumptive patients in Ethiopia. We compared LIODetect TB ST test to Lowenstein Jensen (LJ) culture and GeneXpert MTB/RIF (Xpert) results to determine the diagnostic accuracy.

METHOD

Study Design and Setting: A comparative institutional based cross-sectional study was conducted from June 2018 to July 2019. All presumptive PTB patients, age > = 18 years of age, who were either seen at the Gondar University Comprehensive Specialized Hospital or one of the other three health centers in Gondar Town (Gondar Health Center, Azezo Health Center and Maraki Health Center), were consecutively recruited for this study. Gondar University Comprehensive Specialized Hospital is the only referral and specialized hospital located in Gondar Town, Northwest Ethiopia. This hospital offers a wide range of services including TB diagnosis using Xpert, Löwenstein Jensen (LJ) culture, and phenotypic and genotypic drug susceptibility testing and TB treatment for MDR TB patients.

Subject enrollment and data collection– Socio-demographic data, morning sputum and blood samples were collected from all presumptive PTB (an individual with clinical manifestations consistent with TB)(22). The diagnosis of active TB disease and initiation of treatment was based on the GeneXpert MTB/RIF (Xpert), which is the Ethiopian National TB diagnostic standard test for all hospital level facilities and clinicians' clinical assessment. The clinicians were blinded to the results of the LIODetect TB-ST rapid test and thus the LIODetect results did not influence the diagnosis of TB or the initiation of treatment.

Ethical Statement: Consent was obtained from all participants prior to specimen collection. Samples and collected data were decoded and used only for the intended research. This human subjects protocol was approved by the University of Gondar Ethical Review Board (R. No.-O/V/P/RCS/05/387/2016).

Data and sample Collection - Data were collected using pre-tested structured questionnaires that assess the socio-demographic and clinical characteristics of enrolled participants. For LJ culture and Xpert tests, two morning sputum samples, one for each test, were collected per participant and immediately processed following Ethiopia's TB program standard Operations Procedures for TB diagnosis. Morning sputum samples were collected using two 50 ml plastic tubes (about 5 ml sputum each). Samples were visually checked for quality whether it was saliva or sputum and labeled using patients' medical record number and a unique code. For blood collection, three ml venous blood was collected from each enrolled participant using a plain test tube, allowed to clot, centrifuged, and serum separated.

Xpert test - One ml sputum was mixed with 2 ml sample reagents and vortexed until a clear solution was achieved and left for 15 min to settle. Of this mixture, 2 ml were transferred into the Xpert cartridge using a sterile pasture pipette. Samples were analyzed following the manufacturer' instructions and Ethiopia's TB program standard Operations Procedures(23). Results were available in less than 2 hour and interpreted by the Xpert system automatically.

LJ culture -Sputum samples were decontaminated

and homogenized with *N*-Acetyl L-cysteine- Sodium Hydroxide (NALC-NaOH)(0.5% NALC,4% NaOH and 2.9% sodium citrate). NALC-NaOH solution was freshly prepared prior to use as it is readily inactivated by oxidation. An equal volume of NALC-NaOH solution was added to the sputum and gently mixed for no longer than 30 second. The mixture was allowed standing for 15 min with occasional agitation followed by phosphate buffer saline neutralization (PBS; pH: 6.8) and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the pellet suspended in 2ml of PBS. After vortex mixing, 2-3 drops of this suspension were inoculated into two LJ culture tubes (one glycerol and the other with pyruvate to favor the growth of *M. bovis*), incubated at 37°C, and read once per week for 8 weeks (24, 25) or until a positive growth characteristic of *M. tuberculosis* complex was observed.

LIODetect TB-ST rapid test -This is a lateral flow immuno-chromatographic and membrane based test for the qualitative detection of IgG, IgA and IgM antibodies to *M. tuberculosis* in serum, plasma or whole blood (Lionex diagnostic and therapeutic GmbH, Braunschweig, Germany). This test (Fig. 1) contains a membrane coated (T) with: i) a special antibody binding protein, conjugated to colloidal gold particles (conjugate); ii) three test lines, two lines consisting of two specific recombinant antigens from *M. tuberculosis*, and the third containing a highly purified mycobacterial cell wall antigen (LAM), and iii) a control line ("C") consisting of an antibody binding protein, indicating that the test had been properly performed. Three ml venous blood was collected from each enrolled participant in a tube, allowed to clot for 30 min and centrifuged at 3,000 rpm for 5 min to obtain serum. Following the manufacturer's instructions, 2 drops of the isolated serum were pipetted into the sample well ("S") of the LIODetect TB-ST test cassette followed by2 drops of the LIODetect TB-ST test diluents provided with the test. Results were read after 20 min(21).This test was read as follows: A positive result is noted when two or three pink/purple lines appear. One line should be visible in the control zone ("C") and one or two other lines in the test zone ("T") (Fig.1).The test lines "T" may be stronger or weaker than the control line "C". Very weak shadow-like test lines should be regarded as negative. This test contains an internal positive control(21).When only one colored line appears in the control zone (control line "C") and no visible line (s) in the test zone ("T"), then the test is negative. The test is considered invalid if no control line is visible and/or background color affects readability (21).

HIV testing -HIV testing was done on whole blood using the rapid HIV test algorithm of the Federal Ministry of Health of Ethiopia Wantai/Uni-Gold/ Vikia.

Statistical Analysis - Data were entered in Epi-Data version 3.1 (Epi-Data Association, Odense, Denmark) and analyzed using SPSS vr. 20 (Statistical Package for the Social Sciences, Chicago, IL, USA). Descriptive statistics were employed. Sensitivity, specificity and positive and negative predictive values (PPV, NPV) including 95% confidence intervals (CIs) of the LIODetect TB-ST test were calculated as compared to the Ethiopian TB diagnosis national standards LJ culture and Xpert using SPSS vr. 20 and MedCalc version 11.5.1.0 (MedCalc software, Mariakerke, Belgium), the Kappa coefficient was also generated.

RESULT

A total of 233 presumptive PTB patients were included in this study. The mean age of the enrolled participants was 38.4 years. The majority of the participants were male (n=122, 52.4%) and lived in rural areas (n=125, 53.6%).The majority of these (n=208, 89.3%) were newly diagnosed TB cases, while the rest (n=25, 10.7%) were previously treated TB patients. Of enrolled participants, the majority also were HIV negative (n=190, 81.5%), and the rest were PLWH (n=43, 18.5%)(Table1).

Out of the total233presumptive TB patients,28.3% (n=66)were positive by LJ culture and 25.8% (n=60) were positive by Xpert, while 21.5% (n=50) were positive by LIODetect TB-ST test. The LIODetect TB-ST test showed a sensitivity of 53.0%,a specificity of 91.0%,a PPV of 70.0%,and a NPV of 83.1%,with overall accuracy of 80.3% when compared to LJ culture as our reference method. Conversely, when compared to the Xpert as our reference method, the LIODetect TB-ST test had a sensitivity of 69.6%,a specificity of 90.6%, a PPV of 67.9%, and a NPV of 91.1%, with over all accuracy of 85.8% (Table 2).

The diagnostic performance of LIODetect TB-ST test was also evaluated for detecting TB in PLWH showing a sensitivity of 62.5%, and specificity of 88.6%, a PPV of 55.6%, and a NPV of 91.2%,with overall accuracy of 83.7% when using LJ culture as a reference method. When using Xpert as a reference standard, the LIODetect TB-ST test showed a sensitivity of 55.6%, a specificity of 94.1%, a PPV of 71.4 %, and a NPV of 88.9%, with over all accuracy of 86.1% (Table 3).

Table 1: Socio-demographic characteristics of study participants and positivity of LJ- culture, Xpert *MTB/*RIF and LIODetect TB-ST rapid test TB-ST Tuberculosis Rapid Test

Variable	**LJ Culture**		**Xpert *MTB*/RIF**		**LIODetect TB-ST test**	
	Positive N (%)	**Negative N (%)**	**Positive N (%)**	**Negative N (%)**	**Positive N (%)**	**Negative N (%)**
Sex						
Male	34 (14.6)	88 (37.8)	27(11.6)	95(40.8)	33(14.2)	89(38.2)
Female	32 (13.7)	79 (33.9)	25(10.7)	86(39.6)	20(8.6)	91(39.1)
Age						
0-15 years	0 (0)	8 (3.4)	1 (0.4)	7(3)	0(0)	8(3.1)
16-30 years	37 (15.9)	57 (24.5)	29(12.4)	65(27.9)	24(10.3)	70(30)
31-45 years	19 (8.2)	48 (20.6)	14(6)	53(22.7)	14(6)	53(22.7)
46-60 years	9 (3.9)	35 (15.0)	6(2.6)	38(16.3)	10(4.3)	34(14.6)
>60 years	1 (0.4)	19 (8.2)	2(0.9)	18(7.7)	5(2.1)	15(6.4)
Address						
Urban	30 (12.9)	78 (33.5)	23(9.9)	85(36.5)	26(11.2)	82(35.2)
Rural	36 (15.5)	89 (38.2)	29(12.4)	96(41.2)	27(11.6)	98(42.1)
HIV Status						
Positive	11 (4.7)	32 (13.7)	7(3.0)	36(15.5)	9(3.9)	34(14.6)
Negative	55 (23.6)	135 (57.9)	45(19.3)	145(62.2)	44(18.9)	146(62.7)
TB treatment						
New	56 (24.0)	152 (65.2)	47(20.2)	161(69.1)	44(18.9)	164(70.4)
Previously treated	10 (4.3)	15 (6.4)	5(2.1)	20(8.6)	9(3.9)	16(6.9)

Table 2: Sensitivity, specificity and accuracy of the LIODetect TB-ST test for both people living with HIV (PLWH) and HIV negative people with presumptive pulmonary TB (PTB) against LJ culture and Xpert MTB/RIF reference tests

Reference Methods	LIODetect TB-ST test						
	Positive	Negative	Sensitivity	Specificity	PPV	NPV	Accuracy
LJ Culture							
Positive	35	31	53.0 % (40.3 % to 65.4%)	91.0 % (85.6 % to 94.9%)	70.0 % (57.8 % to 79.9 %)	83.1 % (79.1% to 86.4 %)	80.3 % (74.6 % to 85.2 %)
Negative	15	152					
Xpert							
M.tb Detected	36	17	69.6 % (54.9% to 81.3%)	90.6% (85.4% to 94.4%)	67.9% (56.5% to 77.5%)	91.1% (87.2% to 93.9%)	85.8% (80.7% to 90.1%)
M.tb not detected	16	164					

Notes: *M.tb, Mycobacterium tuberculosis; NPV, Negative Predictive Value; PPV, Positive Predictive Value; Xpert, GeneXpert MTB/RIF*

Table 3: Sensitivity, specificity and accuracy of the LIODetect TB-ST test for people living with HIV (PLWH) with presumptive pulmonary TB (PTB) against LJ culture and Xpert MTB/RIF reference tests

Reference Methods	LIODetect TB-ST test						
	Positive	Negative	Sensitivity	Specificity	PPV	NPV	Accuracy
LJ Culture							
Positive	5	3	62.5 % (24.5 to 91.5%)	88.6 % (73.3 to 96.8%)	55.6% (30.0 to 78.4%)	91.2 % (80.7 to 93.2%)	83.7% (69.3 to 93.2%)
Negative	4	31					
Xpert							
M.tb Detected	5	4	55.6% (21.2% to 86.3 %)	94.12% (80.3% to 99.3%)	71.4 % (36.6% to 91.6%)	88.9% (79.3% to 94.4 %)	86.1% (72.1% to 94.7%)
M.tb not detected	2	32					

Notes: *M.tb, Mycobacterium tuberculosis; NPV, Negative Predictive Value; PPV, Positive Predictive Value; Xpert, GeneXpert MTB/RIF*

DISCUSSION

Delayed diagnosis of TB leads to increased sufferings of patients, transmission of the disease and hampers the TB prevention and control efforts significantly resulting in a public health concern. Currently, available TB diagnostic tools have different limitations: i) Intradermal tuberculin test has low sensitivity and is labor intensive; ii) Acid fast bacilli (AFB) stain microscopy is not sensitive; iii) Culture takes 4-8 weeks; and iv) Polymerase Chain Reaction (PCR) testing is quick but not affordable for low-income settings. Therefore, rapid, cost-effective, simple to use and POC diagnostic tests are urgently needed for effective TB prevention and control, especially in high-burden settings such as Ethiopia. This study evaluated the performance of LIODetect TB-ST POC test perfomrance against Ethiopian current gold standard methods: Xpert and LJ culture,for the diagnosis of PTB.Under our experimental

conditions, the LIODetect TB-ST test had an overall accuracy of 80.3%and 85.8% when compared to LJ culture and Xpert, respectively. This accuracy was slighly enhanced when we analyzed LIODetect TB-ST test performance on PLWH presumptive of PTB, having an accuracy of 83.7% and 86.1%, when compared to LJ culture and Xpert, respectively.

Most diagnostic tests for TB rely on sputum samples, which are problematic to obtain in specific groups of patients like immunocompromised patients, patients with disseminated TB, and children, which leads to delayed treatment initiation, which facilitates TB transmision and disease complications(26).

In our study the sensitivities of LIODetect TB-ST rapid test among presumptive PTB patients compared with LJ and Xpert *MTB*/RIF as gold standard TB diagnosis reference tests were 53.0% and 69.6%, respectively. Those sensitivities were lower than the manufacturer sensitivities reported from both, in house and external studies being 65.4% and 72.8%, respectively (21).This difference is probably due to the gold standard references being used. In our study, we used bacteriologically confirmed methods, while the manufacturer only used clinical diagnosis as a reference method (21). However, the sensitivity of LIODetect TB-ST test detected in our study is comparable to the Alere Determine LAM-Ag test in urine, reported to be at 59% (27). Mean while, our results also showed the specificity of LIODetect TB-ST test being 91.0% and 91.6%, when compared to LJ culture and Xpert. This was comparable with the specificities reported by the manufacturer in their in-house and external studies, which were 96.6% and 94.0%, respectively compared to clinical diagnosis (21). Interestingly, the LIODetect TB-ST also had similar specificity than the other POC Alere Determine LAM-Ag test in urine, being 96% (27).

In our study the sensitivity of the LIODetect TB-ST test for PLWH using LJ culture and Xpert as gold standard reference methods was 62.5% and 55.6%, respectively, which is higher than the reported sensitivity for the Alere Determine LAM-Ag test in urine in several published studies with a range from 37.1% to 44% sensitivity. In contrast, the LIODetect TB-ST test sensitivity in our study was lower than the sensitivity reported for the new POC Fuji LAM test, reported being at 70·4%(28, 29).The specificity of LIODetect TB-ST test for PLWH was 88.6% and 94.1% when compared to LJ culture and Xpert, respectively, which were comparable with the published specificity of the Alere Determine LAM-Ag test ranging from 91% to 97.6% (27, 30-33). The overall accuracy of the LIODetect TB-ST test for PLWH having presumptive PTB in our study was 83.7%. This was similar with the published overall accuracy of the Alere Determine LAM-Ag test being at 79.9% (32).These findings highlight that the LIODetect TB-ST test has a good specificity to identify positive cases as positive, whereas its sensitivity is low.

The low sensitivity showed by this test indicates that could give higher rates of false negative results. Although the sensitivity of the LIODetect TB-ST test was even lower in PLWH, it still performs at an optimal level. Thus, the LIODetect TB-ST low sensitivity should be improved and used as a POC diagnostic test, for both non-HIV individuals and PLWH.

Nonetheless, the LIODetect TB-ST test may offer a benefit over other different diagnostic tests, such as the LJ culture and Xpert as it avoids the need for a productive sputum sample. In this context, and when

compared to other POC Alere Determine LAM-Ag tests, the LIODetect TB-ST test was more valuable for PLWH. Thus, we believe that our results are promising given that the LIODetect-test is a simple POC test that is affordable (less than US$3.0 per test) and quick, providing final results with in 20 min, and interpretation of results is easy. It does also not require electrical/battery power, specialized staff training, and calibration, and requires only 2 drops of whole blood, plasma, or serum (~100 µl), which can be obtained from all patients including children and critically ill patients via a single finger stick and capillary tubes. These factors make it an ideal POC test for use in rural healthcare posts and hospitals in high-burden TB/HIV settings,. However, the LIODetect TB-ST test sensitivity was fairly in both tested groups under our experimental conditions; thus, this test sensitivity needs to be improved to be used as a POC diagnostic test that could significantly reduce the initial loss-to-follow-up in the healthcare cascade (21, 34).

In this context, one of the pillars for decreasing TB incidence is rapid diagnosis. Even today, the lack of appropriate TB diagnosis is the main reason for failure to timely detection of TB, particularly in resource -limited, high burden TB/HIV nations, where it is difficult for established laboratories to perform cultures and other more sophisticated molecular diagnostic tests. (e.g. Xpert). Indeed, in many countries or regions within them, TB diagnosis is still largely based on sputum AFB smears and clinical chest X-rays detection, both of which have low sensitivity and specificity, respectively(27).

Importantly, the LIODetect TB-ST test had better sensitivity and similar specificity in both, HIV negative people and PLWH when compared to the Alere Determine LAM-Ag test reported in other published studieswith similar populations. Thus, the LIODetect TB-ST test, being an antibody-based POC test, could add a beneficial use for TB diagnosis, especially if targeting PLWH and children populations.

Limitation: The limitation of LIODetect TB-ST testis thatit cannot not be used as a drug susceptibility testing and for treatment monitoring, and its sensitivity is suboptimal. Oursample size for PLWH presumptive TB patients was small. A well-controlled healthy group (true negative control) was not included in this study. We didn't include children because of difficulties in obtaining appropriate sputum samples for Culture and GeneXpert. Our results emphasize the potential beneficial use of the LIODetect TB-ST test in PLWH for the diagnosis of PTB. The test requires a finger stick for small amount of blood and can be performed in any setting where getting sputum samples become a challenge.

CONCLUSION

The LIODetect TB-ST test showed excellent specificity when compared to LJ culture and Xpert, as well as to other POC TB diagnosis test published. Although the LIODetect TB-ST test sensitivity was better than the Alere Determine LAM-Ag test performed in urine, it needs to be further improved for TB (pulmonary, extra pulmonary, disseminated) detection in both PLWH and HIV negative people. In resource-limited settings, the LIODetect TB-ST test could be an alternative test especially for PLWH and children who cannot produce productive sputum.

Ethics approval and consent to participate*:* This study protocol was approved by University of Gon-

dar Ethical Review Board (R. No. O/V/P/ RCS/05/387/2016).Consent and assent were obtained from all participants prior to specimen collection. Samples and collected data were decoded and used only for the intended research.

Availability of data and materials*:* Not applicable

Consent for publication*:* Not applicable

Competing interests: The authors declare no competing interest.

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Authors contributions: *WB:* Conceived and designed the study, participated in sample collection, performed laboratory experiments, analyzed and interpreted the data, wrote the first draft and final write up of the manuscript. *JBT:* Analyzed and interpreted the data, edited drafts, approved the final manuscript, and agreed with manuscript results and conclusions. *SW:* Designed the study, analyzed and interpreted the data, edited and approved the final manuscript, and agreed with manuscript results and conclusions. *BT:* Designed the study, analyzed and interpreted the data, edited and approved the final manuscript, and agreed with manuscript results and conclusions.

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REFERENCE

1. WHO. WHO, Global tuberculosis report 2019. 2019.
2. WHO. Global tuberculosis report 2020. 2020.
3. Patrick C, Sheela S. Diagnostics for pulmonary tuberculosis Postgrad Med J 2016;92:187-93.
4. Fakhruzzaman M, Abidin N, Aziz Z, Lim W, Richard J, Noorliza M, et al. Diversified lineages and drug resistance profiles of clinical isolates of Mycobacterium tuberculosis complex in Malaysia. . Int J Mycobacterio. 2019;8:320-8.
5. Adane A, Damena M, Weldegebreal F, Mohammed H. Prevalence and Associated Factors of Tuberculosis among Adult Household Contacts of Smear Positive Pulmonary Tuberculosis Patients Treated in Public Health Facilities of Haramaya District, Oromia Region, Eastern Ethiopia. Tuberculosis Research and Treatment. 2020.
6. M. PL, Jasmine G, G. SS, M. DC, R. SK, Madhukar P. Immunodiagnosis of Tuberculosis: State of the ArtMed Princ Pract 2012;21:4–13.
7. Kwak N, Hwang S, Yim J. Effect of COVID-19 on tuberculosis notification, South Korea. Emerg Infect Dis. 2020.
8. Aung WW, Ei PW, Nyunt WW, Swe TL, Lwin T, Htwe MM, et al. Phenotypic and genotypic analysis of anti-tuberculosis drug resistance in Mycobacterium tuberculosis isolates in Myanmar. Ann Lab Med. 2015;35(5):494-9.

9. Salindri AD, Sales RF, DiMiceli L, Schechter MC, Kempker RR, Magee MJ. Isoniazid Monoresistance and Rate of Culture Conversion among Patients in the State of Georgia with Confirmed Tuberculosis, 2009-2014. Ann Am Thorac Soc. 2018;15(3):331-40.
10. CF M, N M, JM R. et al . The potential impact of COVID-19-related disruption on tuberculosis burden. Eur Respir J. 2020;56(2).
11. Cilloni L, Fu H, Vesga JF, Dowdy D, Pretorius C, Ahmedov S, et al. The potential impact of the COVID-19 pandemic on the tuberculosis epidemic a modelling analysis. . EClinical Medicine -the Lancet 28 2020;28.
12. P G. Predicted impact of the COVID-19 pandemic on global tuberculosis deaths Available from: https://www.medrxiv.org/content/10.1101/2020.04.28.20079582v1.full-text Epidemiology. 2020.
13. García- BAL, A. D, B. S, D.R. S, D. P, GGegia. M, et al. Point of care diagnostics for tuberculosis. Pulmonol. 2018;24(2):73-85.
14. Sen W, Jing W, Jiazhen C, Yan G, Shu Z, Zhou Z, et al. Evaluation of Mycobacterium tuberculosis-specific antibody responses for the discrimination of active and latent tuberculosis infection. International Journal of Infectious Diseases. 2018;70:1-9.
15. Walid B-S, Hedi H, Jalel B. Immunochromatographic IgG/IgM Test for Rapid Diagnosis of Active Tuberculosis. CLINICAL AND VACCINE IMMUNOLOGY 2011;18(12):2090-4.
16. Drain Paul K HKA, Wilson Douglas. A new point-of-care test to diagnose tuberculosis. wwwthelancetcom/infection 2019;19.
17. Tobias B, P. NM, B. SG, Eduardo G, J. ZA, Shireen S, et al. Diagnostic accuracy of 3 urine lipoarabinomannan tuberculosis assay in HIV netative outpatients J Clin Invest 2020;130 (11):5756-64.
18. J T, JB. T. Mannose-capped lipoarabinomannan in Mycobacterium tuberculosis pathogenesis. . Pathog Dis. 2018;76(4).
19. Bjerrum S, Broger T, Székely R, Mitarai S, Opintan JA, Kenu E, et al. Diagnostic accuracy of a novel and rapid lipoarabinomannan test for diagnosing tuberculosis among people with human immunodeficiency virus. . Open Forum Infectious Diseases. 2020;7(1).
20. Esin N, Toyin T, P GM, Rita S, Aurélien M, Dawda J, et al. Comparing accuracy of lipoarabinomannan urine tests for diagnosis of pulmonary tuberculosis in children from four African countries: a cross-sectional study. . Lancet Infect Dis. 2020.
21. Lionex diagnostics and therapeutics LIO-Detect®TB-ST tuberculosis rapid test. REF: 3010_EN Rev. 3.0 / 181019.
22. HEALTH FDROEMO. NATIONAL COMPREHENSIVE TUBERCULOSIS, LEPROSY AND TB/HIV TRAINING MANUAL for HEALTH CARE WORKERS. PARTICIPANTS‘ MANUAL. ADDIS ABABA. 2017.
23. O'Donnell M. Isoniazid Monoresistance: A Precursor to Multidrug-Resistant Tuberculosis? Ann Am Thorac Soc. 2018;15(3):306-7.
24. Jiyong J, Xinyu Y, Jun Y, Liang c. Evaluation of the genoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, china. Infection and Drug Resistance. 2018(11):1627–34.
25. GLI. Mycobacteriology-laboratory-manual. global laboratory Initiative advancing TB diag-

nosis. First Edition. 2014.

26. M.A Bulterys, Wagner B, Redard-Jacot M, Suresh A, Pollock NR, Moreau E, et al. Point-of-Care Urine LAM Tests for Tuberculosis Diagnosis: A Status Update. J Clin Med. 2020;9:111.
27. Shah M, Variava E, Holmes CB, Coppin A, Golub JE, McCallum J, et al. Diagnostic accuracy of a urine Lipoarrabinomannan test for tuberculosis in hospitalized patients in a high HIV prevalence setting. . J Acquir Immune Defic Syndr. 2009;52(2):145.
28. Bjerrum S, Kenu E, Lartey M, Newman M, Addo K, Andersen A, et al. Diagnostic accuracy of the rapid urine lipoarabinomannan test for pulmonary tuberculosis among HIV-infected adults in Ghana–findings from the DETECT HIV-TB study. BMC Infectious Diseases. 2015;15:407.
29. Broger T, Nicol M, Sze´kely R, Bjerrum S, Sossen B, Schutz C, et al. Diagnostic accuracy of a novel tuberculosis point-of-care urine lipoarabinomannan assay for people living with HIV: A meta-analysis of individual in- and outpatient data. PLoS Med. 2020;17(5).
30. Broger T, Sossen B, Toit E, Kerkhoff A, Schutz C, Reipold E, et al. Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study. Lancet Infect Dis. 2019;19:852–61.
31. WHO. Lateral ow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update 2019.
32. Benjamin A, Cavalcante S, Jamal L, Arakaki-Sanchez D, Lima Jd, Pilotto J. Accuracy of Determine TB-LAM Ag to detect TB in HIV infected patients associated with diagnostic methods used in Brazilian public health units. PLoS ONE. 2019;14(9).
33. Lydia N, V M, Yukari C, Mark P, Molly H, Derek T, et al. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. J Acquir Immune Defic Syndr. 2014;66(3):270-9.
34. Songkhla M, Tantipong H, Tongsai S, Angkasekwinai N. Lateral Flow Urine Lipoarabinomannan Assay for Diagnosis of Active Tuberculosis in Adults With Human Immunodeficiency Virus Infection: A Prospective Cohort Study Open Forum Infectious Diseases. 2019.