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Tuberculosis Case Finding With Combined Rapid Point-of-Care Assays (Xpert MTB/RIF and Determine TB LAM) in HIV-Positive Individuals Starting Antiretroviral Therapy in Mozambique

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Background. Tuberculosis is a major health concern in several countries, and effective diagnostic algorithms for use in human immunodeficiency virus (HIV)–positive patients are urgently needed.

Methods. At prescription of antiretroviral therapy, all patients in 3 Mozambican health centers were screened for tuberculosis, with a combined approach: World Health Organization (WHO) 4-symptom screening (fever, cough, night sweats, and weight loss), a rapid test detecting mycobacterial lipoarabinomannan in urine (Determine TB LAM), and a molecular assay performed on a sputum sample (Xpert MTB/RIF; repeated if first result was negative). Patients with positive LAM or Xpert MTB/RIF results were referred for tuberculosis treatment.

Results. Among 972 patients with a complete diagnostic algorithm (58.5% female; median CD4 cell count, 278/μL; WHO HIV stage I, 66.8%), 98 (10.1%) tested positive with Xpert (90, 9.3%) or LAM (34, 3.5%) assays. Compared with a single-test Xpert strategy, dual Xpert tests improved case finding by 21.6%, LAM testing alone improved it by 13.5%, and dual Xpert tests plus LAM testing improved it by 32.4%. Rifampicin resistance in Xpert-positive patients was infrequent (2.5%). Among patients with positive results, 22 of 98 (22.4%) had no symptoms at WHO 4-symptom screening. Patients with tuberculosis diagnosed had significantly lower CD4 cell counts and hemoglobin levels, more advanced WHO stage, and higher HIV RNA levels. Fifteen (15.3%) did not start tuberculosis treatment, mostly owing to rapidly deteriorating clinical conditions or logistical constraints. The median interval between start of the diagnostic algorithm and start of tuberculosis treatment was 7 days.

Conclusions. The prevalence of tuberculosis among Mozambican HIV-positive patients starting antiretroviral therapy was 10%, with limited rifampicin resistance. Use of combined point-of-care tests increased case finding, with a short time to treatment. Interventions are needed to remove logistical barriers and prevent presentation in very advanced HIV/tuberculosis disease.

Keywords. Tuberculosis; HIV; Xpert MTB/RIF; LAM; Africa.

Tuberculosis represents the main cause of death in persons with human immunodeficiency virus (HIV), with a particularly severe burden in resource-limited countries [1–3]. A common integrated model able to deliver concurrently diagnosis and care for both diseases is expected to reduce morbidity and mortality rates significantly [4]. In clinical terms, the diagnosis is more complex in HIV-infected individuals because tuberculosis may present differently, involving extrapulmonary disease with higher frequency [5]. Conventional sputum tests, based on microscopy and culture, are both time and resource demanding, do not identify extrapulmonary disease, and lead to high rates of loss to follow-up and missed treatment [5, 6].

Operationally, there is a need for screening and testing strategies based on point-of-care (POC) tests able to provide rapid and reliable results with good sensitivity [7]. The Xpert mycobacterium tuberculosis/rifampicin (MTB/RIF) assay is a rapid polymerase chain reaction–based assay [8, 9] that detects in clinical samples, within <2 hours, the presence of *Mycobacterium tuberculosis* complex DNA and mutations associated with resistance to rifampin. The test, endorsed by the World Health Organization (WHO) in 2010 [9], requires limited laboratory resources, and can therefore be considered a POC or "near-POC" test, with important operational benefits.

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Recent meta-analyses have shown that in adults with possible tuberculosis, with or without HIV infection, the assay is sensitive and specific and substantially increases tuberculosis detection compared with smear microscopy [10].

The lateral flow urine lipoarabinomannan assay (Determine tuberculosis lipoarabinomannan(TB LAM) assay), another recent POC test, detects lipoarabinomannan, a lipopolysaccharide present in mycobacterial cell walls, in persons with active tuberculosis disease [11]. The WHO identifies the LAM assay as a usable tool to assist in diagnosing tuberculosis in HIV-positive patients who have signs and symptoms of tuberculosis (pulmonary and/or extrapulmonary) or are seriously ill [12]. Although this assay has lower sensitivity than other methods for detecting tuberculosis in adults with HIV, its use in combination with other tests may increase the diagnostic rate in HIV-positive individuals, particularly in those with low CD4 cell counts [11].

The combination of LAM and sputum Xpert assays (along with clinical signs) was associated with a 13% increase in sensitivity over the Xpert assay alone [13]. To define optimal diagnostic algorithms for tuberculosis among persons with HIV, we defined a study protocol based on combined diagnostic testing of sputum (Xpert) and urine (LAM) samples from all HIV-infected persons eligible for antiretroviral therapy (ART). The main objectives of the study were to define the local prevalence of tuberculosis disease (using WHO-endorsed molecular testing that also assess rifampicin resistance) among ART-naive HIV-positive subjects, the prevalence of subclinical disease in patients positive for tuberculosis (using WHO-defined symptoms), and the added values of including in diagnostic algorithms for tuberculosis a repeated Xpert sputum test and a urine LAM test.

METHODS

The study was conducted within the Disease Relief through Excellent and Advanced Means (DREAM) program of the Community of S. Egidio, an Italian faith-based nongovernmental organization. The study protocol was approved by the National Committee for Health Bioethics of the Mozambican Ministry of Health (36; ref. no. CNBS/2014). All participants provided informed consent. The study included all HIV-positive patients >15 years old followed up in the DREAM health centers of Maputo, Machava and Beira (Mozambique) and prescribed ART; patients were screened for tuberculosis before starting ART, with a combined approach that included WHO 4-symptom screening (WHO-4SS; symptoms included fever, current cough, night sweats, and weight loss) [14], a rapid test for detection of mycobacterial lipoarabinomannan in urine (Determine TB LAM assay; Alere), and a molecular tuberculosis assay performed on sputum samples (Xpert MTB/RIF Assay system; Cepheid), which was repeated if the first result was negative.

Demographic and clinical information was collected during routine clinical visits at the DREAM health centers. Participants provided 1 or 2 respiratory sputum samples (with a second sample collected 2 or 3 days later only if the first result was negative), which were tested within 48 hours using the Xpert MTB/RIF Assay system, and 1 urine sample, which was tested within 1 hour using the Determine TB LAM assay; both assays were performed according to manufacturer's instructions. Detection of *M. tuberculosis* was carried out using a 4-module (G4) GeneXpert Dx System, with Xpert MTB/RIF Assay, version 5, and GeneXpert Dx System software, version 4.4a (Cepheid). Xpert testing was performed at Maputo (for the Maputo and Machava centers) and Beira.

Patients with either positive Xpert or positive LAM test results were considered tuberculosis infected and were referred for tuberculosis treatment, usually first-line treatment with a 4-drug fixed drug combination (150-mg rifampicin, 75-mg isoniazid, 400-mg pyrazinamide, and 275-mg ethambutol, once a day administration), with an individually defined second-line treatment when rifampicin resistance was detected with the Xpert assay. All patients were prescribed a combination of tenofovir, lamivudine, and efavirenz, unless clinically contraindicated. Population characteristics were summarized as medians with interquartile ranges (IQRs). Qualitative variables were compared using χ^2 or Fisher tests, and quantitative variables using the Mann-Whitney *U* test. Differences were considered statistically significant at $P < .05$. All analyses were performed using SPSS software, version 22 (IBM).

RESULTS

The study enrollment started in September 2014 and ended in October 2016. Overall, 1015 patients entered the study. Four of them were not eligible because of ongoing treatment for tuberculosis at enrollment (n = 2) or previous ART (n = 2). Thirty-nine additional patients were excluded because did not complete the protocol-defined tuberculosis diagnostic procedures: 7 had no tuberculosis test at all (2 died and 5 dropped out before any test), and 32 had incomplete tuberculosis testing (5 had the LAM test only [3 LAM negative, 2 LAM positive], 1 had the Xpert test only (2 Xpert tests with negative results), and 26 (24 LAM negative, 2 LAM positive) had a first Xpert test with negative results not followed by a second Xpert test (7 died and 19 dropped out).

The remaining 972 patients completed the protocol diagnostic procedures and were included in the analysis. Their general characteristics and sites of enrollment are reported in Table 1. Overall, 98 patients (10.1%) tested positive with either Xpert (n = 90; 9.3%) or LAM (n = 34; 3.5%) assays. Patients who tested positive for tuberculosis were more commonly male, older, and from the clinical site of Beira and showed significantly worse clinical and laboratory parameters, including lower CD4 cell counts and hemoglobin levels, higher plasma HIV RNA levels, and more advanced HIV WHO clinical stage.

Table 1. Population Characteristics and Determinants of Tuberculosis-Positive Status

Characteristic	Patients, No. (%)			*P* value
	All (n = 972)	LAM or Xpert Positive (n = 98)	LAM or Xpert Negative (n = 874)	
Sex				
Male	403 (41.5)	57 (58.2)	346 (39.6)	<.001
Female	569 (58.5)	41 (41.8)	528 (60.4)	
Age, median (IQR), y	35 (30–43)	37 (31–43)	35 (29–43)	<.001
Site of enrollment				
Beira	171	41 (24.0)	130 (76.0)	<.001
Maputo	387	24 (6.2)	363 (93.8)	
Machava	414	33 (8.0)	381 (92.0)	
WHO HIV clinical stage				
I	649 (66.8)	20 (20.4)	629 (72.0)	<.001
II	189 (18.4)	15 (15.3)	174 (19.9)	
III	125 (12.9)	63 (64.3)	62 (7.1)	
IV	9 (0.9)	0 (0)	9 (1.0)	
Plasma HIV RNA, median (IQR), $\log_{10}$ copies/mL (n = 145)	4.18 (3.19–5.04)	5.33 (4.76–5.43)	4.05 (3.13–4.80)	.009
CD4 cell count, median (IQR), cells/μL (n = 971)	278 (142–395)	129 (61–232.5)	292 (160.75–408)	<.001
Hemoglobin, median (IQR), mg/dL (n = 960)	11.8 (10-4-13.2)	9.7 (8.15–11.5)	11.9 (10.7–13.3)	<.001
Presence of ≥1 WHO-4SS symptom				
Yes	334 (34.4)	76 (77.6)	258 (29.5)	<.001
No	638 (65.6)	22 (22.4)	616 (70.5)	
Presence of ≥1 household contact with tuberculosis (n = 863)				
Yes	29 (3.4)	1 (1.6)	28 (3.5)	.43
No	834 (96.6)	61 (98.4)	773 (96.5)	

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; LAM, Determine TB LAM assay; MTB/RIF, Mycobacterium tuberculosis/rifampicin; WHO-4SS, World Health Organization 4-symptom screening; Xpert, Xpert MTB/RIF assay.

At symptom screening with WHO-4SS, 334 patients (34.4%) were positive for ≥1 symptom, with 76 of them (22.8%) positive at either Xpert or LAM testing. Among the 98 patients with positive results of either Xpert or LAM tests, 22 (22.4%) had negative symptom screening results with WHO-4SS. Patients who had ≥1 WHO symptom had significantly lower CD4 cell counts (median, 187/μL; IQR, 82–335/μL) than those with no WHO-4SS symptoms (318/μL; 183.75–418/μL; $P < .001$). In the entire study population, the overall prevalence of tuberculosis among house contacts was 3.4%, with no significant differences between groups (Table 1).

The first Xpert test was positive in 74 of 972 patients (7.6%), and the second Xpert test, performed in the 898 patients with negative results at the first test, identified 16 additional patients, corresponding to 1.8% of those retested after initial negative results. Among 81 Xpert-positive patients with determinable resistance information, 2 (2.5%) had rifampicin resistance.

Concordance between Xpert and LAM test results was low. Among 74 patients with a first positive Xpert result, 24 (32.4%) were concomitantly positive at LAM testing. Among the Xpert-positive patients, LAM-positive patients had significantly lower CD4 cell counts than LAM-negative patients (median, 75/μL vs 149.5/μL; $P = .003$). Overall, LAM-positive patients (n = 33) had significantly lower CD4 cell counts (median 67/μL; IQR, 30–177/μL) than LAM-negative patients (n = 938; 282.5/μL; 149.75–399/μL, $P < .001$). Very few patients who tested negative with the Xpert assay were LAM positive: among 898 patients with a first negative Xpert result, only 10 (1.1%) were LAM positive, and 2 of them had a positive Xpert result when retested, corresponding to 8 patients who were LAM positive only in the entire study (0.8% with a dual-test Xpert strategy) (Table 2).

Fifteen of the 98 patients with positive Xpert or LAM test results did not start treatment for tuberculosis (5 dropped out, 3 transferred [a changed health center or a changed residence], 2 received no treatment owing to a clinician's decision, and 5 died). Among the remaining 83 patients who started treatment for tuberculosis, the median interval between start of the diagnostic algorithm (date of first Xpert test) and the start of tuberculosis treatment was 7 days (IQR, 4–9 days; range, 0–52 days). The median interval from tuberculosis diagnosis to the start of treatment was 6 days (IQR, 3–8 days; range, 0–52 days).

Overall, 24 patients who completed the tuberculosis diagnostics (13 with positive Xpert and/or LAM results) did not start ART, for the following reasons: 4 transferred (2 tuberculosis positive), 2

Table 2. Results of the Tuberculosis Diagnostic Flowchart

First Xpert Result (n = 972)	Patient, No. (%)	LAM Result	N (%)	Second Xpert Result (n = 898)	No (%)
Positive	74 (7.6)	Negative	50 (67.6)	…	…
		Positive	24 (32.4)	…	…
Negative	898 (92.4)	Negative	888 (98.9)	Negative	874 (98.4)
				Positive	14 (1.6)
		Positive	10 (1.1)	Negative	8 (80)
				Positive	2 (20)

Abbreviations: LAM, Determine TB LAM assay; MTB/RIF, Mycobacterium tuberculosis/rifampicin; Xpert, Xpert MTB/RIF assay.

refused ART (0 tuberculosis positive), 10 dropped out (4 tuberculosis positive), and 8 died (7 tuberculosis positive). The remaining 948 patients started a 3-drug combination regimen, most commonly tenofovir, lamivudine, and efavirenz (n = 878; 92.6%) and less commonly zidovudine, lamivudine, and efavirenz (n = 29; 3.1%), zidovudine, lamivudine, and nevirapine (n = 28; 3.0%), or other regimens (n = 13; 1.4%). The median interval between completion of the tuberculosis diagnostic algorithm and the start of ART for the entire population was 6 days (IQR, 3–10 days), with a significant longer interval among tuberculosis-positive compared with tuberculosis-negative subjects (median, 21 vs 5 days, respectively; IQR, 14–29 vs 3–8 days; $P < .001$).

Patients followed up in Beira were significantly more likely to have positive test results for tuberculosis (prevalence, 24.0%, vs 6.2% in Maputo and 8.0% in Machava; $P < .001$ for both comparisons), had a lower median CD4 cell count (182/μL, vs 308/μL in Maputo and 283/μL in Machava; both $P < .001$), and more advanced WHO HIV clinical stage (only 33.3% WHO stage I in Beira, vs 63.0% in Maputo and 84.1% in Machava; both $P < .001$). Patients from Beira were also more commonly male (51.5% vs 39.5% in Maputo [$P = .009$] and 39.1% in Machava [.006]), with no differences in median age (34 vs 35 and 36 years, respectively; $P > .05$ for both comparisons).

DISCUSSION

With the current WHO approach, based on administration of ART to all HIV-infected individuals, irrespective of CD4 cell count, ART can be started immediately after HIV testing in HIV-positive individuals, but it is critical to define their tuberculosis status, because tuberculosis may have severe consequences in terms of morbidity and mortality rates. In low- or medium-income countries, symptom screening is often used, but a significant proportion of patients with tuberculosis may be symptom negative and therefore be missed by simple symptom screening [15]. Identification of tuberculosis in culture requires time and resources, and even simpler approaches based on chest radiography or identification of acid-fast bacilli in sputum, although more rapid, may be operationally problematic. Implementation of rapid and reliable tests is therefore essential for an adequate diagnosis and treatment of tuberculosis in subjects with HIV.

Within this context, we evaluated a "reinforced" WHO-endorsed approach, in which a first Xpert test was accompanied by a urine LAM test and an additional Xpert test, when the result of the first Xpert test was negative. With this approach, we observed a 10.1% prevalence of tuberculosis in an unselected population of HIV-infected individuals starting ART. In similar studies conducted in Africa, the prevalence of cultured *M. tuberculosis* from sputum in HIV-infected individuals at the start of ART was slightly higher (17%–18%) [15–17], whereas in selected samples from other African countries, the prevalence was similar or even lower (13% in a large Ugandan HIV clinic [18], 6% in Ethiopian symptomatic patients mostly receiving ART [19], and 2.4% in pregnant women from Kenya [20]).

In our study, prevalence showed marked geographic differences, with a 24% prevalence in Beira (the second-largest town, located in central Mozambique), compared with 6.2% in the capital Maputo and 8.0% in the smaller town of Machava, both in Southern Mozambique. The higher prevalence in Beira was not due to case selection, because all patients eligible for ART were included in the study, and it might therefore indicate higher local circulation of the disease in the population. Patients from Beira were in a more advanced disease stage, had lower CD4 cell counts, and were more frequently male. All these conditions increase the risk of tuberculosis in individuals with HIV [21–23] and may have represented additional cofactors. Mozambique is among the 22 high-burden countries for tuberculosis [24], and distinct characteristics of the tuberculosis epidemics in the Beira district have already been described [25]. Particular attention should be directed toward the tuberculosis epidemics in this district, where prevalence and disease burden among individuals with HIV seem to be greater.

Rifampicin resistance, based on the results of the Xpert test, was relatively rare (2.5%). Previous studies had shown high rates of drug resistance in Mozambique; in samples sent for drug susceptibility testing, mostly from pretreated subjects, rates as high as 58.3% were reported [26, 27]. In subjects with no previous tuberculosis treatment, resistance was usually less frequent: 2 studies from the same region had shown rifampicin resistance in 5%–6% of the samples analyzed [28, 29]. The present findings, therefore, showing a 2.5% resistance rate to rifampicin, can be considered reassuring.

The prevalence of tuberculosis among house contacts was low (3.4%) and strictly consistent with data from a large systematic review, which showed a 3.1% prevalence of active

tuberculosis among household contacts [30]. Patients with tuberculosis showed significant differences from tuberculosis-negative patients for some characteristics, which generally represent already well-described tuberculosis predictors: male sex, older age, more advanced HIV disease, and lower CD4 cell count and hemoglobin levels [21, 23, 31, 32]. The concurrence of tuberculosis with advanced HIV disease and the high mortality rate in the few days after enrollment suggest that an earlier diagnosis of HIV and tuberculosis could prevent significant disease and death. In general, the interval from tuberculosis diagnosis to the start of tuberculosis treatment was short (median, 6 days), but several patients did not start treatment, mostly owing to transfer, dropout or rapidly deteriorating clinical conditions. This situation represents an important operational challenge [28, 33].

The current study provided information on the additional value in tuberculosis case finding of adding a repeated Xpert test and a urinary LAM test to a single Xpert test. The second Xpert test identified as tuberculosis positive 16 additional patients with initially negative Xpert results (17.8% of all Xpert-positive cases and 16.3% of all tuberculosis cases detected), improving case finding by 21.6% compared with a single-test Xpert strategy. This is fully consistent with the 20% increase in sensitivity produced by a second sampling in children [34] and with the 22.9% increase reported in adults when a dual-test Xpert strategy was used [16]. Similarly, LAM testing identified 10 additional patients with negative at the first Xpert test (10.2% of all tuberculosis cases detected), and 8 patients with negative results at 2 consecutive Xpert tests (8.1% of all tuberculosis cases detected), improving case finding by 13.5% compared with a single-test and by 8.9% compared with a dual-test Xpert strategy. Such figures are consistent with the incremental diagnostic yield of 13.4% compared with Xpert testing and clinical signs only, reported in hospitalized or severely ill HIV-infected individuals from Kenya [13].

Overall, the combined addition of a LAM test and a second Xpert test enabled identification of 24 additional cases (24.5% of all tuberculosis cases detected), improving case finding by 32.4% compared with a single-test Xpert strategy. This represents a significant advantage, which should be weighed against the additional costs of the procedures. The transfer of this strategy to any clinical setting and all economic evaluations, however, needs to take into account that tuberculosis case finding per se does not necessarily translates into clinical benefits, if tuberculosis treatment is not effectively implemented. In this study, 15% of tuberculosis cases did not receive tuberculosis treatment, confirming that a significant proportion of the patients with tuberculosis diagnosed do not start treatment [28, 33]. Efforts should be directed to prevent this occurrence, reducing as much as possible logistical difficulties and barriers to treatment, and preventing through earlier testing the presentation of patients with extremely advanced disease and very poor clinical conditions, significant predictors of both death and attrition [35].

The data support the evaluation of tuberculosis in all HIV-positive patients at presentation, irrespective of symptoms: a symptom-based screening is likely to miss a significant proportion of tuberculosis cases, as already reported [16, 20], with important clinical consequences for patients with undiagnosed tuberculosis, who are likely to present at a later stage, when treatment response is lower and mortality rates higher [35]. The persisting reliance on symptoms is documented by a few cases in which clinicians decided not to start tuberculosis treatment despite a positive test result (LAM or Xpert positive in 1 patient each) because patients were asymptomatic and in good general condition. This occurrence clearly underlines the difficulties associated with the introduction of rapid laboratory tests as the only diagnostic tools in a setting where empirical treatment based on clinical evaluation only had been widely used [36, 37].

The external validity of the study is provided by a population-based recruitment that included all the HIV-infected individuals eligible for ART (unselected group). This study did not evaluate the presence of tuberculosis using conventional tests such as radiography, sputum microscopy, and sputum culture, but LAM and Xpert tests have been evaluated extensively in comparison with such diagnostic techniques [11, 34, 38–40]. Moreover, although the Xpert test has been already validated and endorsed for tuberculosis diagnosis, with widespread use in several African countries, information on its field application was still limited, and the current study contributed information that may be useful in implementing screening programs in similar settings.

In summary, using a combined diagnostic approach based on rapid POC tests, we showed a 10% prevalence of undiagnosed tuberculosis in a large and unselected series of HIV-infected Mozambican individuals starting ART, with a significant added diagnostic value of repeated Xpert testing and LAM urinary testing. Symptom screening was only partially effective in identifying tuberculosis-positive patients. The diagnostic algorithm confirmed the feasibility of a rapid tuberculosis diagnostic process in the field, but roughly 15% of patients with tuberculosis diagnosed did not receive tuberculosis treatment because of very severe disease or for logistical reasons. Further research and interventions are needed to remove logistical barriers to tuberculosis treatment and prevent presentation of patients with very advanced HIV/tuberculosis disease.

Notes

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References

1. UNAIDS. Fact sheet November 2016: global HIV statistics. **2016**. Available at: http://www.unaids.org/en/resources/fact-sheet. Accessed 4 April 2017.
2. World Health Organization. Global tuberculosis report 2016. **2016**. Available at: http://www.who.int/tb/publications/global_report/en/. Accessed 4 April 2017.

3. Manosuthi W, Wiboonchutikul S, Sungkanuparph S. Integrated therapy for HIV and tuberculosis. AIDS Res Ther **2016**; 13: 22.
4. Ford N, Matteelli A, Shubber Z, et al. TB as a cause of hospitalization and in-hospital mortality among people living with HIV worldwide: a systematic review and meta-analysis. J Int AIDS Soc **2016**; 19: 20714.
5. Wasserman S, Meintjes G. The diagnosis, management and prevention of HIV-associated tuberculosis. S Afr Med J **2014**; 104:886–93.
6. Wallis RS, Maeurer M, Mwaba P, et al. Tuberculosis-advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis **2016**; 16:e34–46.
7. Scott L, da Silva P, Boehme CC, Stevens W, Gilpin CM. Diagnosis of opportunistic infections: HIV co-infections—tuberculosis. Curr Opin HIV AIDS **2017**; 12: 129–38.
8. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol **2010**; 48: 229–37.
9. World Health Organization. Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva, Switzerland: World Health Organization; **2011**.
10. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev **2014**; 1:CD009593.
11. Shah M, Hanrahan C, Wang ZY, et al. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in HIV-positive adults. Cochrane Database Syst Rev **2016**; 5: CD011420.
12. World Health Organization. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV: policy guidance. **2015**. Available at: http://www.who.int/tb/publications/use-of-lf-lam-tb-hiv/en/. Accessed 24 March 2017.
13. Huerga H, Ferlazzo G, Bevilacqua P, et al. Incremental yield of including Determine-TB LAM assay in diagnostic algorithms for hospitalized and ambulatory HIV-positive patients in Kenya. PLoS One **2017**; 12:e0170976.
14. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Geneva, Switzerland: World Health Organization, **2011**.
15. Henostroza G, Harris JB, Chitambi R, et al. High prevalence of tuberculosis in newly enrolled HIV patients in Zambia: need for enhanced screening approach. Int J Tuberc Lung Dis **2016**; 20: 1033–9.
16. Lawn SD, Brooks SV, Kranzer K, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. PLoS Med **2011**; 8:e1001067.
17. Naidoo K, Karim QA, Bhushan A, et al. High rates of tuberculosis in patients accessing HAART in rural South Africa. J Acquir Immune Defic Syndr **2014**; 65: 438–46.
18. Roy M, Muyindike W, Vijayan T, et al. Implementation and operational research: use of symptom screening and sputum microscopy testing for active tuberculosis case detection among HIV-infected patients in real-world clinical practice in Uganda. J Acquir Immune Defic Syndr **2016**; 72: e86–91.
19. Adelman MW, Tsegaye M, Kempker RR, et al. Intensified tuberculosis case finding among HIV-infected persons using a WHO symptom screen and Xpert(®) MTB/RIF. Int J Tuberc Lung Dis **2015**; 19: 1197–203.
20. LaCourse SM, Cranmer LM, Matemo D, et al. Tuberculosis case finding in HIV-infected pregnant women in Kenya reveals poor performance of symptom screening and rapid diagnostic tests. J Acquir Immune Defic Syndr **2016**; 71: 219–27.
21. Auld AF, Mbofana F, Shiraishi RW, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy—Mozambique, 2004–2008. PLoS One **2013**; 8: e54665.
22. García-Basteiro AL, López-Varela E, Respeito D, et al. High tuberculosis burden among people living with HIV in southern Mozambique. Eur Respir J **2015**; 45: 547–9.
23. Pathmanathan I, Dokubo EK, Shiraishi RW, et al. Incidence and predictors of tuberculosis among HIV-infected adults after initiation of antiretroviral therapy in Nigeria, 2004–2012. PLoS One **2017**; 12: e0173309.
24. World Health Organization. Tuberculosis in Mozambique. **2017**. Available at: http://www.who.int/countries/moz/areas/tuberculosis/en/. Accessed on 13 April 2017.
25. Saifodine A, Fyfe J, Sievers A, Coelho E, Azam K, Black J. Genetic diversity of *Mycobacterium tuberculosis* isolates obtained from patients with pulmonary tuberculosis in Beira city, Mozambique. Int J Mycobacteriol **2014**; 3: 94–100.
26. Pires GM, Folgosa E, Nquobile N, Gitta S, Cadir N. *Mycobacterium tuberculosis* resistance to antituberculosis drugs in Mozambique. J Bras Pneumol **2014**; 40:142–7.
27. Namburete EI, Tivane I, Lisboa M, et al. Drug-resistant tuberculosis in Central Mozambique: the role of a rapid genotypic susceptibility testing. BMC Infect Dis **2016**; 16: 423.
28. Cowan J, Michel C, Manhiça I, et al. Implementing rapid testing for tuberculosis in Mozambique. Bull World Health Organ **2015**; 93: 125–30.
29. Valencia S, Respeito D, Blanco S, et al. Tuberculosis drug resistance in Southern Mozambique: results of a population-level survey in the district of Manhiça. Int J Tuberc Lung Dis **2017**; 21: 446–51.
30. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J **2013**; 41: 140–56.
31. Liu E, Makubi A, Drain P, et al. Tuberculosis incidence rate and risk factors among HIV-infected adults with access to antiretroviral therapy. AIDS **2015**; 29: 1391–9.
32. Kerkhoff AD, Wood R, Vogt M, Lawn SD. Predictive value of anemia for tuberculosis in HIV-infected patients in Sub-Saharan Africa: an indication for routine microbiological investigation using new rapid assays. J Acquir Immune Defic Syndr **2014**; 66: 33–40.
33. Bos JC, Smalbraak L, Macome AC, Gomes E, van Leth F, Prins JM. TB diagnostic process management of patients in a referral hospital in Mozambique in comparison with the 2007 WHO recommendations for the diagnosis of smear-negative pulmonary TB and extrapulmonary TB. Int Health **2013**; 5: 302–8.
34. Rachow A, Clowes P, Saathoff E, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. Clin Infect Dis **2012**; 54: 1388–96.
35. Molfino L, Kumar AM, Isaakidis P, et al. High attrition among HIV-infected patients with advanced disease treated in an intermediary referral center in Maputo, Mozambique. Glob Health Action **2014**; 7: 23758.
36. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? Lancet Infect Dis **2014**; 14:527–32.
37. Bacha JM, Ngo K, Clowes P, et al. Why being an expert—despite Xpert—remains crucial for children in high TB burden settings. BMC Infect Dis **2017**; 17: 123.
38. Theron G, Zijenah L, Chanda D, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet **2014**; 383: 424–35.
39. Ho J, Nguyen PT, Nguyen TA, et al. Reassessment of the positive predictive value and specificity of Xpert MTB/RIF: a diagnostic accuracy study in the context of community-wide screening for tuberculosis. Lancet Infect Dis **2016**; 16: 1045–51.
40. Lawn SD. Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infect Dis **2012**; 12: 103.