

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The World Health Organization Recommended TB Diagnostic Tools

Lynn S. Zijenah

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.73070>

Abstract

Tuberculosis (TB) is one of the top 10 causes of death worldwide. TB has further been exacerbated by the HIV/AIDS pandemic, the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant TB. In 2015, approximately 1.4 million people and 400,000 who were HIV-negative and HIV-positive, respectively, died of TB. There were 10.4 million new cases with active TB of which 2.4 million were HIV co-infected and 480,000 new cases with MDR-TB. Conclusions: TB is a multifaceted disease and there is no one size fits all test for its diagnosis. In the 22 high TB burden countries (HTBBC), which harbour 80% of global TB, sputum smear microscopy with its low detection rate remains the most commonly used diagnostic test for pulmonary TB. Culture, the gold standard for TB diagnosis, the molecular-based tests for both rapid diagnosis and detection of drug resistant TB because of the requirement for specialized laboratories and trained personnel as well as other costs is not routinely used in most HTBBC. An accurate, affordable, point-of-care TB test, with no requirement for electricity, specialized laboratory, easily performed by healthcare personnel is what is urgently needed for TB control.

Keywords: sputum smear microscopy, TB-LAMP, LAM-LF, culture, MTB/RIF assay, line-probe assays

1. Introduction

Tuberculosis (TB), one of the top 10 major causes of death globally is a major public health priority. Of the 10.4 million people diagnosed with active TB in 2015, the majority occurred in people living in low- and middle-income countries. The TB epidemic has further been exacerbated by the HIV/AIDS pandemic and the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. Of the estimated 1.8 million people who died in 2015, 1.4

million were HIV negative and 400,000 were HIV positive. In the same period, 480,000 cases of MDR TB and a further 100,000 that were estimated to be rifampicin-resistant [1].

TB mainly affects the lungs (pulmonary TB), however, it can affect other parts of the body such as the spine or brain (extrapulmonary TB). Although TB is a preventable and curable disease, failure to detect the disease early is one of the major bottlenecks to TB control. TB diagnostic tests with low sensitivity that were developed more than a century ago are still in use today. The detection case rates in the 22 high burden countries which harbor 80% of global TB burden is low (~50%) and even lower among the HIV-infected. [2]. From the 10.4 million people who developed TB in 2015, 4.3 million cases were not diagnosed or notified and only one quarter of MDR TB cases (132,000) were detected and reported. The reasons for the low detection and underreporting of TB are multifactorial and include limited or delayed access to appropriate diagnosis and care, large private sectors not reporting cases, and the lack of access to appropriate diagnostic tools due to geographic and/or financial barriers [3–5].

The WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) provides objective, ongoing technical and strategic advice to the WHO regarding TB diagnosis, care and control (http://www.who.int/tb/advisory_bodies/stag/en/index.html). The group which is comprised of 22 experts representing ministries of health, national TB control programs, academic and research institutions, civil society organizations, communities and patients affected by TB, and professional associations provides the WHO Director General with independent evaluations of the strategic, scientific, and technical aspects of WHO's area of work in TB. The group also reviews progress and challenges in TB-related core functions such as policies, strategies, and standards and make recommendations on committees and working groups. The STAG-TB reviews policy drafts and supporting documentation and may endorse the policy recommendation with or without revisions, request additional information and re-review the evidence in subsequent years, or reject the recommendation.

Below, we describe the WHO recommended TB diagnostic tools, the advantages and disadvantages as well as challenges in the implementation of the tools.

2. World Health Organization (WHO) approved TB diagnostic tools

2.1. Sputum smear microscopy for the diagnosis of pulmonary TB

Direct microscopic examination of sputum for acid-fast bacilli (AFB), the sputum smear microscopy (SSM) remains the most commonly used diagnostic test for pulmonary TB particularly in countries with a high rate of TB infection [6].

The test is conducted by placing a thin layer of the sputum (smear) on a glass slide. A series of special stains are then applied to the smear, and the stained slide is examined under a microscope for signs of the TB bacteria [7]. It is a simple inexpensive test which does not

require sophisticated laboratory infrastructure or extensive training of laboratory personnel and the results are available within hours. Although its sensitivity is only about 50–60%, its high specificity (99–100%) ensures that only those who are positive receive the anti-TB treatment [8]. The detection rate is even lower in countries with a high prevalence of both pulmonary TB and HIV infection, as many patients with HIV and TB co-infection have very low levels of TB bacteria and are unable to produce good quality sputum leading to false negative results [9].

SSM has other limitations in addition to its low sensitivity. False positive results may occur in individuals that have been infected with NTB. False negative results particularly happen with children, older people and HIV-infected patients. Furthermore, SSM cannot be used to diagnose extrapulmonary TB. Many HIV-infected patients tend to have high rates of extrapulmonary TB compared to HIV negative individuals which probably contributes to the lower sensitivity of FM in this group of patients [9].

In the earlier years, a conventional light microscope for examining the AFB Ziehl-Neelsen (ZN) stains was recommended for SSM in low-income and middle-income countries where most of the world's TB cases occur [10, 11]. In high-income countries, AFB auramine O or auramine-rhodamine stains are examined by fluorescence microscopy (FM) which has a higher sensitivity than conventional ZN light microscopy. In these countries, FM is the most commonly used method for diagnosis of pulmonary TB [12].

In FM, the smear is illuminated with a quartz halogen or high pressure mercury vapor lamp, allowing a much larger area of the smear to be seen and resulting in more rapid examination of the specimen. The major advantage of FM is that it uses a lower power objective lens compared to conventional microscopy thus reducing the time of assessing the same area of a slide [13]. The major disadvantage of using FM is the expensive mercury vapor lamp which lasts a very short time. The lamp also takes a while to warm up, burn high amounts of electricity, and electricity supply problems can significantly shorten its life span [14]. The use of light emitting diodes (LEDs) which switch on extremely quickly, have an extremely long life, and do not explode can address some of these problems [14].

In 2006, a systematic review of 45 studies comparing conventional SSM with FM reported that FM has a higher sensitivity than the standard light microscopy but similar specificity with standard light microscopy [15]. In HIV positive patients, there was insufficient data to determine the value of FM.

Following a systematic review in September 2009, of a meta-analysis of published and unpublished data, the WHO assessed the evidence for the efficacy of LED microscopy. Subsequently in 2011 the WHO issued a policy statement recommending that conventional FM should be replaced by LED microscopy [16].

The advantages of LED microscopes are: they are less expensive, require less power and are able to run on batteries, the bulbs have a very long half-life and do not pose the risk of releasing potentially toxic products if broken.

In 2011, the WHO revised its earlier recommendations of using three sputum specimens collected on different days to same day microscopy using two sputum specimens collected at the same time, on the same day based on a systematic review of 37 eligible studies [17, 18]. However, the WHO recommended its use only in settings with a well-established laboratory network and a fully functional external quality assurance program for SSM including on-site evaluation and follow-up training for problem laboratories.

The revised recommendation has reduced the number of patient visits to the clinic, leading to a reduction in the numbers of TB positive cases that are lost to follow up, reduced laboratory workload as well as decreased time for diagnosis and initiation of anti-TB treatment with non-significant decrease in diagnostic yield [19].

2.2. Loop-mediated isothermal amplification (TB-LAMP) for diagnosis of pulmonary TB

A commercial molecular assay Loopamp MTBC Detection Kit was developed by Eiken Chemical Company Ltd. (Tokyo, Japan) for the detection of MTBC (TB-LAMP) [20].

The assay is based on loop-mediated isothermal amplification. It is a manual assay that requires less than 1 h to perform and the result can be read with the naked eye under ultra violet (UV) light. The assay consists of three steps, sample preparation (10–20 min), amplification (40 min), and visual detection of fluorescence light from the reaction tube using UV light (0.5–1 min) (**Figure 1**). Sputum is added to a heating tube containing the extraction solution which is then mixed by inverting, the heating tube is placed into the heating block to lyse and inactivate mycobacteria. The heating tube is then removed from the heating block and allowed to cool. The heating tube is then attached to an adsorbent tube and mixed by shaking until all the powder has been completely mixed with the solution. An injection cap is placed onto the adsorbent tube and screwed tightly to pierce the seal. The nozzle is then inserted into a reaction tube and drops of solution are transferred to the reaction tube. Amplification is carried out by loading the reaction tubes into the heating block and the reaction started. The amplification is stopped automatically after 40 min. For visual detection of fluorescent light, the reaction tubes are transferred into a fluorescence detector and the results recorded [21, 22].

The TB-LAMP assay has several features that makes it attractive as a diagnostics platform for resource-poor settings: it is fast (40 min), isothermal (requiring only a heat block), robust to inhibitors and reaction conditions that usually adversely affect polymerase chain reaction (PCR) methods, and it generates a result that can be detected with the naked eye. The major disadvantage of TB-LAMP is that it cannot detect drug resistance and is therefore only suitable for testing of patients at low risk of MDR TB [22].

In January 2016, WHO Guideline Development Group (GDG) conducted a systematic review and meta-analysis of 24 studies conducted after 01 January 2012 to evaluate the use of TB-LAMP on sputum samples from adults with signs and symptoms consistent with pulmonary TB that were conducted in settings with an intermediate or high burden of TB. Only 13

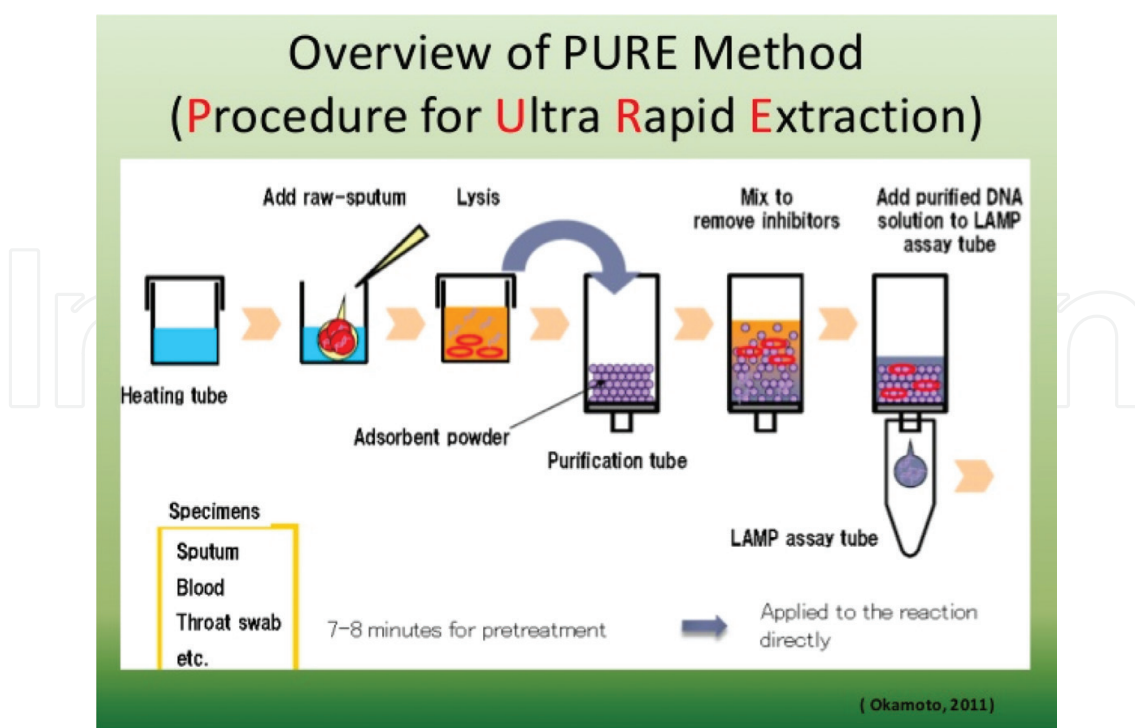


Figure 1. Overview of Pure method (procedure for ultra-rapid extraction [21]).

of the 24 studies met the eligibility criteria for inclusion in the systematic review. The pooled sensitivity of TB-LAMP was higher than that of SSM (78% vs. 63%). The pooled specificity of TB-LAMP was lower than that of SSM, 98% vs. 100%. In the HIV-infected patients, the pooled sensitivity of TB-LAMP was similar to that of SSM; 64% vs. 62% for SSM while specificity was the same, 99% for TB-LAMP and 99% for SSM. In the analysis of TB-LAMP for detection of pulmonary TB in adult patients who were SSM negative, TB-LAMP showed a 42% incremental yield [23].

In August 2016, WHO issued a policy recommendation on the TB-LAMP MTBC assay. TB-LAMP may be used as a replacement test for SSM to diagnose pulmonary TB in adults with signs and symptoms consistent with TB and TB-LAMP may be used as a follow-on test in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary. These recommendations apply to settings where conventional SSM can be performed, TB-LAMP should not replace the use of rapid molecular tests that detect TB and resistance to RIF, especially among populations at risk of MDR TB. Due to the limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over SSM for testing persons living with HIV who have signs and symptoms consistent with TB. These recommendations are extrapolated to using TB-LAMP in children, based on the generalization of data from adults, while acknowledging the difficulties of collecting sputum specimens from children. TB-LAMP should not replace the Xpert MTB/RIF assay because the Xpert MTB/RIF assay can detect resistance to RIF whilst the former cannot [23].

2.3. Gene Xpert MTB/RIF assay

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), is an automated semi-quantitative nested real-time PCR for the rapid detection of MTBC DNA and RIF resistance simultaneously, directly from unprocessed sputum within 2 h [24]. The assay has been extensively evaluated in various geographical settings and the diagnostic accuracy is good [25–31]. In a meta-analysis, the pooled sensitivity and pooled specificity of MTB/RIF were 88 and 95% respectively when used as an initial test for TB diagnosis. The pooled sensitivity was 80% in the HIV-infected patients. The pooled sensitivity and pooled specificity for detection of RIF resistance were 94 and 98% respectively. Thus, it was concluded that the MTB/RIF assay is sensitive and specific as an initial test for diagnosis of TB, TB associated HIV and MDR TB [32].

The assay is very simple to run and can be performed by nurses with very little training [33]. Briefly, the assay is carried out by adding the sample reagent in a volume twice that of the untreated sputum and the mixture incubated for 15 min. Two millimeters of the processed sputum is then transferred to the MTB/RIF assay cartridge and then inserted into the Gene Xpert instrument, subsequent steps in the assay are completely automated and self-contained. The advantages of the assay are its higher sensitivity when compared to SSM and shorter period (2 h) of obtaining the result when compared with culture which although it gives a definite diagnosis, it takes weeks. Furthermore the assay identifies RIF resistance within hours compared to the weeks taken to get any drug resistance result when using culture-based methods [34].

In 2011, WHO issued a policy statement recommending the use of the assay as a diagnostic tool for all people living with HIV who have signs and symptoms of TB, for people with unknown HIV status presenting with strong clinical evidence of HIV infection, for people who are seriously ill and suspected of having TB regardless of HIV status and those at risk of MDR TB [35].

Although it has been touted as a new test which represents a major milestone for global TB diagnosis and care and new hope for the millions of people who are at the highest risk of TB and drug-resistant disease, it has some disadvantages [36]. The disadvantages include the short shelf life of the cartridges (only 18 months), very stable electricity supply is required, the instrument needs to be recalibrated annually, and the cost of the test and the temperature ceiling is critical [37]. To address some of these challenges, a new machine, the Xpert Omni which is intended for point of care (POC) testing for TB and RIF resistance, using the same cartridges as those used in the current Xpert machine is currently under development. It is expected that it will be smaller, lighter and less expensive than the current Xpert machine and will also come with a built-in 4 h battery [38].

A next generation cartridge called the GeneXpert Ultra (Ultra) was launched on 24 March 2017, World TB Day [39]. Its sensitivity is higher than that of MTB/RIF with the greatest sensitivity gains being recorded among SSM negative-culture positive patients, and HIV infected-TB patients. It however, has a lower specificity than the MTB/RIF assay. The performance of the Ultra was assessed in 2016 in a multicentre non-inferiority study at 10 sites in 8 low- and middle-income countries. The performance of the Ultra assay was evaluated by the WHO Technical Experts Group in January 2016, which concluded that the Ultra test performed better than the MTB/RIF assay in TB diagnosis of children, HIV-infected patients and patients with extra pulmonary TB, who more often than not are difficult to diagnose, however, there is a need for more research to be conducted to improve the specificity of the new test [40].

The usefulness of the MTB/RIF has generated a lot of controversy. Some people consider the test to be extremely useful, as well as cost effective, and should be used in as many places as soon as possible while other people consider it not to be really suitable and practical at the present time for major use in low- and middle-income countries [41–43]. A clinical trial conducted in four African countries in 2013 comparing the use of the Xpert to SSM concluded that using the Xpert meant that more patients had a same day diagnosis and same day treatment initiation, but the benefits did not translate into lower TB morbidity [33].

In spite the negatives concerning the usefulness of the MTB/RIF in so far as the outcomes of its use are concerned, its introduction since 2010 has revolutionized TB diagnostics as a POC test offering rapid TB diagnosis and simultaneous detection of RIF resistance. More than 23 million Xpert machines had been procured in 130 countries and MDR TB diagnosis more than tripled by 2016 [44, 45].

2.4. Lipoarabinomannan urine strip test for TB diagnosis in HIV-infected patients

A POC lateral flow urine Lipoarabinomannan strip test (LF-LAM) developed by Alere Determine™ TB LAM Ag, Waltham, MA, USA for TB diagnosis is based on the detection of mycobacterial lipoarabinomannan (LAM) antigen in urine. Briefly 60 µL of freshly collected urine is applied to the test strip, incubated at room temperature for 25 min and the result recorded as negative if there was no presence of any band or recorded as positive and band graded using the manufacturer's reference card with bands of graded intensity. The LF-LAM test has been evaluated for accuracy of TB diagnosis in HIV-infected patients in various geographical settings [46–53] albeit with widely varying sensitivity (13–93%) and specificity (87–99%) [53]. The general consensus then was that the assay is most suitable for HIV-infected patients with CD4 counts < 200 cells/µL [54]. The variability in the performance characteristics of LF-LAM led the end users of the assay to request the WHO for guidance on the appropriate use of the assay.

In 2015, the WHO commissioned a systematic review of the use of LF-LAM assay for the diagnosis and screening of active TB in people living with HIV. The quantitative meta-analysis included 16 studies. Following the meta-analysis, the WHO recommended that the LF-LAM test may be used to assist in the diagnosis of TB in HIV positive adult in patients with signs and symptoms of TB (pulmonary and/or extra pulmonary) who have a CD4 cell count less than or equal to 100 cells/µL, or HIV positive patients who are seriously ill regardless of CD4 count or with unknown CD4 count [55]. This recommendation also applies to HIV positive children with signs and symptoms of TB (pulmonary and/or extra pulmonary) based on the generalization of data from adults while acknowledging very limited data and concern regarding low specificity of the LF-LAM assay in children [55].

The advantages of LAM include use of urine which is easily and rapidly obtained even from very ill patients compared to sputum, it is an easy to use POC test which can also be performed by trained nurses making it an ideal POC test. Its major disadvantage is that its use is restricted to a subgroup of HIV-infected TB suspects with low CD4+ T lymphocytes. The reasons for higher sensitivity and specificity in this group of patients are not fully understood. However, it is hypothesized that HIV patients with advanced immunosuppression may have a disseminated TB infection that is very difficult to rapidly diagnose with current tools. The

patients may have a higher bacterial load associated with widespread infection and, therefore, antigen load, the greater likelihood of genitourinary tract TB and greater glomerular permeability to allow increased antigen levels in urine [55].

2.5. Culture for TB diagnosis and drug resistance testing

Culture remains the gold standard for TB diagnosis and drug-resistant testing. Ideally culture examinations should be done on all diagnostic specimens, regardless of AFB smear or nucleic acid amplification results. Positive cultures for MTB confirm the diagnosis of TB disease; however, in the absence of a positive culture, particularly in RLCs, TB disease may also be diagnosed on the basis of clinical signs and symptoms alone. Two types of broth culture systems; liquid and solid media are commercially available. The commercial liquid culture systems and molecular line-probe assays have been endorsed by the WHO as gold standards for rapid detection of MDR TB [56, 57].

The drug resistance of clinical isolates as determined by conventional methods (e.g., broth-based and agar proportion) is due to the presence of mutations in specific MTB genes [58]. These mutations often are single base pair changes in the DNA sequence of the bacteria. There are a variety of commercial assays and laboratory tests that can detect mutations associated with drug resistance. The assays are done on patient specimens or isolates from patient specimens. The liquid based systems such as BACTEC, MGIT, VersaTREK and MBBACT allow detection of most mycobacterial growth in 4–14 days compared to 3–6 weeks for solid media [59]. However these tests require specialized laboratories and skills that are often unavailable in the regions, particularly RLS, where most cases of TB and MDR TB occur [59].

As an interim solution while capacity for genotypic or automated liquid culture and drug sensitivity testing (DST) is being developed, in 2011, the WHO recommended non-commercial culture and DST methods for screening patients at risk for MDR TB namely (i) microscopic observation of drug susceptibility (MODS): a micro colony direct method in liquid culture, based on inoculation of specimens into drug-free and drug-containing media, followed by microscopic examination of early growth, (ii) colorimetric redox indicator (CRA): a direct or indirect method based on the ability of MTB to reduce nitrate, which is detected by a color reaction and (iii) nitrate reductase assay (NRI) methods: indirect methods based on the reduction of a colored indicator added to liquid culture medium on a microtitre plate after exposure of MTB strains to anti-TB drugs *in vitro*. These tests can only be used in reference laboratories and under strict laboratory protocols. The major disadvantage of these tests is that none can detect XDR TB and thus cannot replace the conventional culture and DST tests [60].

2.6. Molecular line-probe assays for diagnosis of TB and detection of drug resistance

The emergence of MDR TB and XDR TB threatens to reverse the gains that have been made in global control of TB. Rapid tests for detection of resistance to anti-TB treatment are urgently required for timeous and appropriate treatment which would lead to decreased morbidity and mortality as well as curbing new infections.

The standard first line drugs for anti-TB treatment include RIF and INH. In patients with MDR TB, drugs belonging to fluoroquinolones (FLQ) and second line injectable drugs (SLID) are used. The FLQ drugs include ofloxacin, levofloxacin, moxifloxacin and gatifloxacin while the SLID include kanamycin (KAN), amikacin (AMK) and capreomycin (CAP) [61]. Patients with XDR TB are resistant to RIF, INH, plus any FLQ and at least one of the three SLIDs thus making them resistant to both first line and second line anti-TB drugs [61].

Turnaround time (TAT) for DST results using the conventional solid based methods ranges from 8 to 12 weeks [62] thus contributing to new infections as those infected continue to transmit drug-resistant TB. On 26 March 2007, the WHO recommended the use of liquid culture and DST in low- and medium-income countries [58] following evidence provided by the Foundation for Innovative New Diagnostics (FIND). Although the liquid media based tests such as BACTEC® (BD Diagnostics, Sparks, MD, USA), MGIT® (BD Diagnostics) and BacT/ALERT® (bioMe'rieux SA, Marcy l'Etoile, France) have a shorter TAT, they are more expensive, require specialized laboratories and trained laboratory personnel [63].

Nucleic acid amplification molecular methods offer several advantages over the conventional culture-based methods which include rapid diagnosis and standardized testing.

In 2005 a meta-analysis of one of the two commercially line-probe assays (LiPAs) that were available then; the INNO-LiPA Rif.TB (Innogenetics, Ghent Belgium) [64] was conducted.

The INNO-LiPA Rif.TB (LiPA) test simultaneously detects MTBC and a mutation in the *rpoB* gene associated with RIF resistance. The test involves extraction of DNA from cultures or directly from clinical specimens, amplification of the RIF resistance-determining region of the *rpoB* gene using PCR, hybridization of the biotinylated PCR products with immobilized probes and determination of results by color-metric development [64].

The meta-analysis to evaluate the accuracy of LiPA for RIF resistance detection comprised of 15 studies which comprised 11 studies that used culture isolates, 1 study that used clinical isolates and 3 studies that used both [64]. The sensitivity and specificity were greater than 95 and 100% respectively in 12 of the 14 studies that used culture isolates in the LiPA test. In the 4 studies that used clinical isolates in the LiPA test, sensitivity ranged between 80 and 100% whilst specificity was 100%. The authors concluded that although LiPA is a highly sensitive and specific test for detection of RIF resistance in culture isolates because of the lower sensitivity when used directly on clinical specimens, more evidence is required before the test can be used to detect MDR TB among populations at risk in clinical practice [64].

In 2008 a meta-analysis of the second LiPA commercially available in the early 2000, the Genotype MTBDR (Hain Life Sciences, GmbH, Nehren Germany) was performed [65]. The Genotype MTBDR (MTBDR) test detects the mutations in the *rpoB* and *katG* genes associated with RIF and isoniazid (INH) resistance respectively. The meta-analysis included 10 published articles contributing 14 comparisons and 15 comparisons for detection of RIF and INH respectively. The pooled sensitivity and specificity for RIF resistance across all the subgroups was 91.1 and 98.7% respectively. The pooled specificity for detection of INH resistance was 99.5%, but the sensitivity was variable and inconsistent, 84.3% (95% CI:76.6–89.8). Ling et al.

concluded that MTBDR assay also referred to as MTBDRsl Version 1 (now referred to simply as Hain version 1) has excellent accuracy for RIF resistance detection. Although specificity for detection of INH resistance was excellent, the sensitivity was modest and variable [65].

In 2008, following the two meta analyses to assess the diagnostic accuracy of LiPA and MTBDR assays, the WHO recommended the use of these LiPAs for MTBC and RIF resistance detection in sputum smear-positive specimens (direct testing) and in cultured isolates of MTBC (indirect testing) [66]. Since then, newer versions of the two LPAs have been developed and a third one, Nipro NTM + MDRTB detection kit 2 (Tokyo, Japan) which detects MTBC, RIF and INH resistance has been introduced.

The FIND evaluated the Nipro and the Hain version 2 LPAs and compared them with Hain version 1 in 2015. The study reported that these three LPAs showed equivalence for detecting TB and resistance to RIF and INH [67].

An updated systematic review of the accuracy of the three LiPAs (Hain version 1, Hain version 2 and Nipro) for detecting MTBC and resistance to RIF and INH was commissioned by the WHO in 2015. The review included 74 studies comprising 94 unique datasets of which 83 datasets evaluated Hain version 1, 5 evaluated Hain version 2, and 6 evaluated the Nipro assay. Subsequently, the WHO in 2016, issued a Policy update on the use of molecular LPAs for the detection of resistance to INH and RIF [68]. The mutation probes used for detection of RIF resistance (*rpoB*), high level INH resistance (*katG*), and low-level isoniazid resistance (*inhA*) are the same for the three assays with the exception of the *katG* S315N mutation, which is included in the Nipro assay but not in Hain version 1 or version 2 [68].

Hain version 1 was developed to genotype resistance to FLQ via *gyrA*, SLID resistance (SLID including KAN, AMK and CAP) via *rrs* and ethambutol (EMB) resistance via *embB*. Hain version 2 also targets *gyrA* but includes assays for *gyrB* mutations that are also associated with FLQ resistance. Furthermore, the assay incorporates further SLID resistance genotypes via the *eis* promoter region. The *embB* resistance component is not used in the Hain version 2.0 [69].

Subsequent to the meta-analysis, the WHO recommended that the commercial molecular LiPAs may be used as the initial test instead of phenotypic culture-based DST to detect RIF and INH in persons (children and adults) with a sputum smear-positive specimen (direct testing) or a cultured isolate of MTBC (indirect testing). However, the accuracy of detecting resistance to RIF and INH differs leading to an overall reduced accuracy of MDR TB diagnosis. LiPAs are not recommended to replace conventional culture-based DST, which may still be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance. Furthermore, when the LiPA result does not detect INH resistance, conventional culture-based DST for INH may still be used to evaluate patients particularly for populations with a high pre-test probability of resistance to INH [68].

2.7. TB skin test for diagnosis of latent TB infection

Latent tuberculosis infection (LTBI) is defined as a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* (MTB) antigens without evidence of clinically manifested active TB. LTBI will lead to active TB disease in approximately 5–10% of these

individuals during their lifetimes; [70], the risk is higher in younger children [71], the immunocompromised or immunosuppressed [72, 73], and in people from countries with a high incidence of TB (≥ 40 cases per 100,000) [74].

Diagnosis of LTBI is important as those found positive may be initiated on prophylactic treatment, thus preventing development of active TB and indirectly preventing transmission for those found to have disease and commenced on anti-TB treatment.

The TB Skin test (TST) is one of the oldest diagnostic tests developed in the 19th century but which is still being widely used [75]. The standard recommended test is the Mantoux test, which is administered by intradermal injection of a 0.1 mL of liquid containing 5 tuberculin units (TU) of purified protein derivative (PPD) or 2 TU PPD RT23 (these are considered equivalent) into the top layers of skin of the forearm. The test is read 48–72 h after the injection [76]. Although widely used the test has several limitations; a positive reaction may be observed in both latent and active TB infection, therefore, it is unreliable in differentiating whether the person is currently having TB or had been infected in the past or at carrier stage; false positive reactions may occur which could be attributed to infection with non-tuberculosis mycobacteria (NTM), previous Bacillus Calmette Guérin (BCG) vaccination [77], incorrect method of TST administration, incorrect interpretation of reaction, incorrect bottle of antigen used; false negative reactions due to cutaneous anergy, recent TB infection (within 8–10 weeks of exposure), very old TB infection, very young age (less than 6 months old), recent live-virus vaccination (e.g., measles and smallpox), overwhelming TB disease, some viral illnesses (e.g., measles and chicken pox) [78, 79]. Thus, a confirmatory test such as sputum culture, is usually done to rule out an active TB infection.

HIV-infected patients may have a compromised ability to respond to the TST because of cutaneous anergy [80, 81]. Tuberculin skin testing assesses the ability to mount a delayed type hypersensitivity (DTH) cell mediated immune response to PPD. Since in HIV infection, there is a gradual decrease in CD4⁺ T lymphocytes, as HIV disease progresses, the HIV-infected patients tend to have an impaired DTH response, which plausibly may cause a false negative TST result.

The TST, however can be used for differential diagnosis of TB from sarcoidosis, another granulomatous disease with similarities to TB. Whilst TST has a high sensitivity for sarcoidosis, it has been reported to have a poor specificity for TB. In the general population, a negative TST is a specific test for sarcoidosis, in contrast, a positive TST in a sarcoidosis patient is a specific test for indicating TB. Thus a thorough TB workup should be done in a sarcoidosis suspect patient [82].

In 2015, the WHO strongly recommended the TST for diagnosis of latent TB in high- and upper medium-income countries with low TB burden (estimated TB incidence less than 100 per 100,000), in the HIV-infected patients, adult and child contacts of pulmonary TB cases, patients initiating anti-tumor necrosis factor treatment, patients receiving dialysis, patients preparing for organ or haematologic transplantation, and patients with silicosis [83].

2.8. Interferon gamma release assays for diagnosis of latent TB infection

The Interferon gamma release assays (IGRAs) measure, using an enzyme-linked immunosorbent assay (ELISA) or an enzyme-linked immunospot (ELISPOT) the release of Interferon- γ (IFN- γ) from T lymphocytes following stimulation of the cells with MTB-specific antigens

There are two commercially available IGRAs: the QuantiFERON® TB Gold (Cellestis Ltd., Carnegie, Victoria, Australia) and the T-SPOT® TB IGRAs (Oxford Immunotec, Oxford, United Kingdom).

In the first-generation QuantiFERON-TB assay, whole-blood is stimulated with PPD and ELISA used to measure the concentration of IFN- γ released by the T lymphocytes [84]. The enhanced form of the assay, the QuantiFERON-TB Gold uses the MTB-specific antigens: early-secreted antigenic target 6-kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10) instead of PPD [84]. In the newer version of the assay, QuantiFERON-TB Gold In-Tube, heparinized venous blood is added to the tube coated with the MTB-specific antigens; ESAT-6, CFP-10, and TB 7.7 [84].

In the T SPOT-TB assay, peripheral blood mononuclear cells are stimulated with ESAT6 and CFP10 and the released IFN- γ detected using an ELISPOT assay [85].

These assays are not routinely used in Resource-Limited Settings (RLS) because they are expensive, require expensive equipment and advanced technical expertise.

In 2015, the WHO recommended the use of IGRAs for the diagnosis and treatment of LTBI in people living with HIV, adult and child contacts of pulmonary TB cases, patients initiating anti-tumor necrosis factor treatment, patients receiving dialysis, patients preparing for organ or haematologic transplantation, and patients with silicosis, prisoners, healthcare workers, immigrants from high TB burden countries, homeless persons and illicit drug users and in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100,000 [76].

The advantages of the IGRAs include only a single patient visit to conduct the TB test, the results can be available within 24 h, and prior BCG vaccination does not cause a false positive result. The disadvantages include; the requirement to process the collected blood specimen fairly rapidly (within 8–16 h following blood collection), laboratory facilities are required, and the test is only for latent TB. Furthermore, the IGRAs may not be as accurate in people who are HIV-infected [72].

2.9. Chest radiography

Chest X-rays (CXR) are not considered as specific diagnostic tests for TB. However, because of the low sensitivity of SSM in TB diagnosis of HIV-infected patients, in 2007, the WHO recommended use of CXRs in HIV-infected patients who are SSM negative [86]. The chest X-ray plays an important role in the diagnosis of TB among people living with HIV and can also be an important entry point to diagnosing non-tubercular chest diseases, which are common among people living with HIV. CXR presentations of TB in HIV-infected patients are now well characterized and CXR play a significant role in shortening delays in diagnosis and should be performed early in the course of investigation of a tuberculosis suspect [86]. Indeed in a randomized controlled trial of Xpert MTB/RIF versus SSM conducted in four countries in southern Africa, the majority of the HIV-infected patients in the SSM arm who were smear-negative were commenced on anti-TB treatment based on radiological findings with or without clinical symptoms, whilst awaiting results from culture [33].

In 2016, the WHO published a factsheet and issued new recommendations and guidance on the use of chest radiography for TB detection in National TB care [87, 88]. CXR may play an essential role as a sensitive tool in diagnosis of childhood pulmonary and extra-pulmonary TB and in excluding active TB prior to treatment of LTBI. Since CXR on their own cannot establish a TB definite diagnosis, in an algorithm of TB screening that involves TB symptoms screening, CXR can also be used as a sensitive tool for screening for active TB, this may improve the pre-test probability of the subsequent diagnostic test and lead to a reduction in the number of people who need to undergo further diagnostic evaluation [89]. CXR is also used in TB prevalence surveys as it is considered the most sensitive screening tool for identifying those survey participants with a high probability of having TB. An abnormal CXR and or positive symptom screen is then followed by bacteriological confirmation [90].

The limitations in the wider use of chest X-rays, include non-availability at peripheral health facilities and the difficulty of interpreting results, even by trained physicians.

3. Conclusion

The upsurge of one the oldest known infectious diseases, TB, coupled with the HIV/AIDS pandemic, emergence of MDR TB and XDR TB has led to unprecedented efforts in developing new TB diagnostic tools which can detect TB and resistance to first line and second line anti-TB drugs more rapidly. Whilst SSM remains the most used tool in diagnosis of pulmonary TB in the majority of countries that harbor the highest burden of TB, new molecular-based amplification techniques, LiPAs which provide results faster have been developed. However, these tests are not available to the majority of the countries with the highest TB burden mainly because of costs, requirement of specialized laboratory and trained personnel with the exception of the Xpert MTB/RIF assay which can be performed by any healthcare giver after minimum training.

The urgent need for development of a true POC TB test with operational simplicity similar to the rapid HIV antibody POC test, which is accurate, easy to use, does not require a laboratory, laboratory trained personnel, nor electricity and is affordable cannot be overemphasized if the Global Strategy and Targets for Tuberculosis Prevention, Care and Control goal of eliminating TB as a public health threat by 2030 is to be achieved.

Acknowledgements

This publication, TESA II RegNet2015-1051 is part of the EDCTP2 Programme, which is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation.

Author details

Lynn S. Zijenah

Address all correspondence to: lzijenah@gmail.com

Department of Immunology, University of Zimbabwe College of Health Sciences, Harare, Zimbabwe

References

- [1] WHO. Global Tuberculosis Report 2016. Geneva, Switzerland: WHO Press; 2016. www.who.int/tb/publications/global_report/en/ [Accessed: 20 August 2017]
- [2] Peter JG, Theron G, Pooran A, Thomas J, Pascoe M, Dheda K. Comparison of two methods for acquisition of sputum samples for diagnosis of suspected tuberculosis in smear-negative or sputum scarce people: A randomised controlled trial. *The Lancet Respiratory Medicine*. 2013;**1**:471-478
- [3] Subbaraman R, Nathavitharana RR, Satyanarayana S, et al. The tuberculosis cascade of care in India's public sector: A systematic review and meta-analysis. *PLoS Medicine*. 2016;**13**:e1002149
- [4] Cazabon D, Alsdurf H, Satyanarayana S, et al. Quality of tuberculosis care in high burden countries: The urgent need to address gaps in the care cascade. *International Journal of Infectious Diseases*. 2017;**56**:111-116
- [5] Wells WA, Uplekar M, Pai M. Achieving systemic and scalable private sector engagement in tuberculosis care and prevention in Asia. *PLoS Medicine*. 2015;**12**:e1001842
- [6] Parsons LM, Somoskövi Á, Gutierrez C, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: Challenges and opportunities. *Clinical Microbiology Reviews*. 2011;**24**:314-350
- [7] Sputum Gram stain—Overview. University of Maryland Medical Center www.umm.edu/ency/article/. [Accessed: 05 September 2017]
- [8] Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: The current evidence. *The Lancet Infectious Diseases*. 2003;**3**:288-296
- [9] Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: Informing urgent policy changes. *Lancet*. 2007;**369**:2042-2049
- [10] Harries AD, Maher D, Nunn P. An approach to the problems of diagnosing and treating adult smear-negative pulmonary tuberculosis in high-HIV-prevalence settings in sub-Saharan Africa. *Bulletin of the World Health Organization*. 1998;**76**:651-662

- [11] Foulds J, O'Brien R. New tools for the diagnosis of tuberculosis: The perspective of developing countries. *The International Journal of Tuberculosis and Lung Disease*. 1998;**2**: 778-783
- [12] Shinnick TM, Iademarco MF, Ridderhof JC. National plan for reliable tuberculosis laboratory services using a systems approach. Recommendations from CDC and the association of public health laboratories task force on tuberculosis laboratory services. *The Morbidity and Mortality Weekly Report*. 2005;**54**(RR-6):1-12
- [13] Toman K. What are the advantages and disadvantages of fluorescence microscopy? In: Frieden T, editor. *Toman's Tuberculosis: Case Detection, Treatment, and Monitoring-Questions and Answers*. 2nd ed. Geneva: World Health Organization; 2004. pp. 31-34
- [14] Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: A systematic review. *The Lancet Infectious Diseases*. 2006;**6**: 570-581
- [15] TB diagnosis: Improving the yield with fluorescence microscopy. 2007. www.aidsmap.com/TB-diagnosis-Improving-the-yield-with-fluorescence-microscopy/. [Accessed: 05 September 2017]
- [16] Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis. WHO; 2011. http://www.who.int/tb/publications/2011/led_microscopy_diagnosis_9789241501613/en/ [Accessed: 05 September 2017]
- [17] WHO. *Same-day Diagnosis of Tuberculosis by Microscopy*. Geneva: World Health Organization; 2011
- [18] Mase SR, Ramsay A, Ng V, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: A systematic review. *The International Journal of Tuberculosis and Lung Disease*. 2007;**11**:485-495
- [19] Cuevas LE, Yassin MA, Al-Sonboli N, et al. A multi-country non-inferiority cluster randomized trial of frontloaded smear microscopy for the diagnosis of pulmonary tuberculosis. *PLoS Medicine*. 2011;**8**:e1000443
- [20] Boehme CC, Nabeta P, Henostroza G, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. *Journal of Clinical Microbiology*. 2007;**45**:1936-1940
- [21] Mitarai S, Okumura M, Toyota E, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. *The International Journal of Tuberculosis and Lung Disease*. 2011;**15**:1211-1217
- [22] Ou X, Li Q, Xia H, Pang Y, Wang S, et al. Diagnostic accuracy of the PURE-LAMP test for pulmonary tuberculosis at the county-level Laboratory in China. *PLoS One*. 2014;**9**:e94544
- [23] *The Use of Loop-mediated Isothermal Amplification (TB-LAMP) for the Diagnosis of Pulmonary Tuberculosis: Policy Guidance*. Geneva: World Health Organization; 2016

- [24] Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampicin resistance. *The New England Journal of Medicine*. 2010;**363**:1005-1015
- [25] Lawn SD, Nicol MP. Xpert® MTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiology*. 2011;**6**:1067-1082
- [26] Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: A multicentre implementation study. *Lancet*. 2011;**377**:1495-1505
- [27] Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for the rapid diagnosis of tuberculosis and detection of rifampicin resistance in pulmonary and extrapulmonary specimens. *Journal of Clinical Microbiology*. 2011;**49**:4138-4141
- [28] Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *Journal of Clinical Microbiology*. 2011;**49**:1202-1205
- [29] Ligthelm LJ, Nicol MP, Hoek KGP, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from needle-aspiration biopsy specimens. *Journal of Clinical Microbiology*. 2011;**49**:3967-3970
- [30] Tortoli E, Russo C, Piersimoni C, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *The European Respiratory Journal*. 2012;**40**:442-447
- [31] Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *American Journal of Respiratory and Critical Care Medicine*. 2011;**184**:132-140
- [32] Steingart KR, Sohn H, Schiller I, et al. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database of Systematic Reviews*. 2013;**1**:1-131
- [33] 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-setting in Africa: A multicentre, randomised, controlled trial. *Lancet*. 2014;**383**:424-435
- [34] Zijenah LS, Bandason T, Gwambiwa B, et al. Integration of point-of-care Xpert MTB/RIF and smear microscopy for TB diagnosis with point-of-care HIV tests, CD4 counts and treatment at a primary health care clinic in Harare, Zimbabwe. *South African Respiratory Journal*. 2014;**20**:104-111
- [35] WHO. Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System: Policy Statement. Geneva, Switzerland: World Health Organization; 2011. http://www.who.int/tb/features_archive/xpert_rapid_tb_test/en/. [Accessed: 02 September 2017]
- [36] Batz H. Towards Lab Free Tuberculosis Diagnosis. August: Access Campaign Medecins Sans Frontieres; 2011 www.msfaccess.org/ Accessed 05 September 2017

- [37] Trébucq A, Enarson DA, Chiang CY, et al. Xpert MTB/RIF for national tuberculosis programmes in low income countries: When, where and how? *The International Journal of Tuberculosis and Lung Disease*. 2011;**15**:1567-1572
- [38] Denkinger CM, Nicolau I, Ramsay A, Chedore P, Pai M. Are peripheral microscopy centres ready for next-generation molecular tuberculosis diagnostics? *The European Respiratory Journal*. 2013;**42**:544-547
- [39] 2017 launch of new TB test Ultra backed by WHO recommendation. www.cepheid.com/us/about-us/news-events/press-releases/ [Accessed: 05 September 2017]
- [40] WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. <http://apps.who.int/iris/handle/10665/254792> [Accessed: 05 September 2017]
- [41] Singh JA, Anant B. The ethics of national tuberculosis programmes in low income countries not rolling out Xpert MTB/RIF. *The International Journal of Tuberculosis and Lung Disease*. 2011;**15**:1563-1563
- [42] Hanrahan CF, Selibas K, Deery CB, et al. Time to treatment and patient outcomes among TB suspects screened by a single point-of-care Xpert MTB/RIF at a primary care clinic in Johannesburg, South Africa. *PLoS One*. 2011;**8**:e66421
- [43] Evans C. GeneXpert—A game changer for tuberculosis control? *PLoS Medicine*. 2011;**8**:e1001064
- [44] World Health Organization website. WHO Monitoring of Xpert MTB/RIF Roll-Out. http://www.who.int/tb/areas-of-work/laboratory/status_xpert_rollout_dec_2016.pdf. [Accessed: 05 September 2017]
- [45] Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme C. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: What lessons have we learnt and how can we do better? *The European Respiratory Journal*. 2016;**48**:516-525
- [46] Peter J, Theron G, van Zyl-Smit R, Haripersad A, Mottay L, Kraus S, et al. Diagnostic accuracy of a urine lipoarabinomannan strip test for TB detection in HIV-hospitalised patients. *The European Respiratory Journal*. 2012;**40**:1211-1220
- [47] Lawn SD, Kerhoff AD, 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. *The Lancet Infectious Diseases*. 2012;**12**:201-209
- [48] Balcha TT, Winqvist N, Sturegard E, et al. Detection of lipoarabinomannan in urine for detection of active tuberculosis among HIV-positive adults in Ethiopian health centres. *Tropical Medicine & International Health*. 2014;**19**:734-742
- [49] Drain PK, Losina E, Coleman SM, et al. 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
- [50] Manabe YC, Nonyane BAS, Nakyingi L, et al. Point-of-care lateral flow assays for tuberculosis and cryptococcal antigenuria predict death in HIV infected adults in Uganda. *PLoS One*. 2014;**9**:e101459

- [51] Shah M, Ssengooba W, Armstrong D, et al. Comparative performance of urinary lipoarabinomannan assays and Xpert MTB/RIF in HIV-infected individuals with suspected tuberculosis in Uganda. *AIDS*. 2014;**28**:1307-1314
- [52] Nakiyingi L, Moodley VM, Manabe YC, et al. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. *Journal of Acquired Immune Deficiency Syndromes*. 2014;**66**:270-279
- [53] Zienah LS, Kadzirange G, Bandason T, et al. Comparative performance characteristics of the urine lipoarabinomannan strip test and sputum smear microscopy in hospitalized HIV-infected patients with suspected tuberculosis in Harare, Zimbabwe. *BMC Infectious Diseases*. 2016;**16**:20
- [54] Minion J, Leung E, Talbot E, Dhedha K, Pai M. Diagnosing tuberculosis with urine lipoarabinomannan systematic review and meta-analysis. *The European Respiratory Journal*. 2011;**38**:1398-1405
- [55] WHO. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV severely HIV-infected patients with no available CD4 counts
- [56] WHO. WHO policy statement. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis. 2008. http://www.who.int/tb/features_archive/policy_statement.pdf [Accessed: 02 September 2017]
- [57] WHO. Use of liquid TB culture and DST in low and medium income settings. http://www.who.int/tb/laboratory/use_of_liquid_tb_culture_summary_report.pdf?ua=1 [Accessed: 05 September 2017]
- [58] Chaudhary M, Gupta S, Khare S, Lal S. Diagnosis of tuberculosis in an era of HIV pandemic: A review of current status and future prospects. *Indian Journal of Medical Microbiology*. 2010;**28**:281-289
- [59] Wilson ML. Recent advances in the laboratory detection of mycobacterium tuberculosis complex and drug resistance. *Clinical Infectious Diseases*. 2011;**52**:1350-1355
- [60] World Health Organization. Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis: policy statement; 2011
- [61] Theron G, Peter J, Richardson M, Warren R, Dheda K, Steingart KR. GenoType® MTBDR#sl assay for resistance to second-line anti-tuberculosis drugs. *Cochrane Database of Systematic Reviews*. 2016;**9**:CD010705
- [62] Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technology Assessment*. 2007;**11**:1-196
- [63] Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *Journal of Clinical Microbiology*. 2004;**42**:2321-2325

- [64] Morgan M, Kalantri S, Flores L, Pai M. A commercial line probe assay for the rapid detection of rifampicin resistance in mycobacterium tuberculosis: A systematic review and meta-analysis. *BMC Infectious Diseases*. 2005;**5**:62
- [65] Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multi-drug-resistant tuberculosis: A meta-analysis. *The European Respiratory Journal*. 2008; **32**:1165-1174
- [66] Rossau R, Traore H, De Beenhouwer H, et al. Evaluation of the INNO-LiPA Rif.TB assay, a reverse hybridization assay for the simultaneous detection of mycobacterium tuberculosis complex and its resistance to rifampin. *Antimicrobial Agents and Chemotherapy*. 1997;**41**:2093-2098
- [67] Report for WHO: non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDRplus V2 line probe assays. Geneva: FIND; 2015. http://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf [Accessed: 04 September 2017]
- [68] World Health Organization, WHO. The Use of Molecular Line Probe Assays for the Detection of Resistance to Second-Line Anti-Tuberculosis Drugs. Geneva: World Health Organization; 2016, 2016. <http://www.who.int/tb/WHOPolicyStatementSLIPA.pdf> Accessed: 05 September 2017
- [69] Hain Life Sciences. Rapid Diagnosis of Tuberculosis Brochure. <http://hain-lifescience.de/uploadfiles/file/produkte/mikrobiologie/mykobakterien/tbeng.pdf>. [Accessed: 23 December 2015]
- [70] Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon- γ release assays and tuberculin skin testing for progression from latent TB infection to disease state: A meta-analysis. *Chest*. 2012;**142**:63-75
- [71] Kasprovicz VO, Churchyard G, Lawn SD, Squire SB, Lalvani A. Diagnosing latent tuberculosis in high-risk individuals: Rising to the challenge in high-burden areas. *The Journal of Infectious Diseases*. 2011;**204**(Suppl 4):S1168-S1178
- [72] Santin M, Munoz L, Rigau D. Interferon- γ release assays for the diagnosis of tuberculosis and tuberculosis infection in HIV-infected adults: A systematic review and meta-analysis. *PLoS One*. 2012;**7**(3):e32482
- [73] Chkhartishvili N, Kempker RR, Dvali N, et al. Poor agreement between interferon-gamma release assays and the tuberculin skin test among HIV-infected individuals in the country of Georgia. *BMC Infectious Diseases*. 2013;**13**:513
- [74] Public Health England. World Health Organization (WHO) estimates of tuberculosis incidence by rate, 2012 (sorted by rate). 2014. <https://www.gov.uk/government/publications/tuberculosis-tb-by-country-rates-per-100000-people>. [Accessed: 03 September 2017]
- [75] Menzies D. Tuberculin skin testing. In: Reichman LB, Hershfield ES, editors. *Tuberculosis: A Comprehensive International Approach*. New York: Marcel Dekker; 2000. pp. 279-322
- [76] Nayak S, Acharjya B. Mantoux test and its interpretation. *Indian Dermatology Online Journal*. 2012;**3**:2-6

- [77] Chaturvedi N, Cockcroft A. Tuberculosis screening among health service employees: Who needs chest X-rays? *Occupational Medicine (London)*. 1992;**42**:179-182
- [78] American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *American Journal of Respiratory and Critical Care Medicine*. 2000;**161**: 1376-1395
- [79] American Thoracic Society/Centers for Disease Control. Targeted tuberculin testing and treatment of latent tuberculosis infection. *American Journal of Respiratory and Critical Care Medicine*. 2000;**161**:S221-S247
- [80] Markowitz N, Hansen NI, Wilcosky TC, et al. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. *Annals of Internal Medicine*. 1993;**119**:185-193
- [81] Graham NMH, Nelson KE, Solomon L, et al. Prevalence of tuberculin positivity and skin test anergy in HIV-1 seropositive and -seronegative intravenous drug users. *Journal of the American Medical Association*. 1992;**267**:369-373
- [82] Smith-Rohrberg D, Sharma SK. Tuberculin skin test among pulmonary sarcoidosis patients with and without tuberculosis: Its utility for the screening of the two conditions in tuberculosis-endemic regions. *Sarcoidosis, Vasculitis, and Diffuse Lung Diseases*. 2006;**23**:130-134
- [83] WHO. Guidelines on the Management of Latent Tuberculosis Infection. Geneva: World Health Organization; 2015. http://www.who.int/tb/publications/lftbi_document_page/en/
- [84] Cellestis. Quantiferon-TB1 Gold. 2009. www.cellestis.com/. [Accessed: 05 September 2017]
- [85] Oxford Immunotec. T.Spot-TB1. 2009. www.oxfordimmunotec.com/ [Accessed: 05 September, 2017] http://www.who.int/tb/publications/2011/mdr_tb_diagnostics_9789241501620/en/ [Accessed: 01 September 2017]
- [86] Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents. Recommendations for HIV-prevalent and resource-constrained settings. World Health Organization; 2007 (WHO/HTM/TB/2007.379); <http://www.who.int/tb/publications/tb-diagnosishiv-recommendations/en/> [Accessed: 5 September 2017]
- [87] Summary of Current WHO Recommendations and Guidance on Programmatic Approaches. Geneva: World Health Organization; 2016
- [88] World Health Organization. Radiography in Tuberculosis Detection. Summary of Current WHO Recommendations and Guidance on Programmatic Approaches. Geneva: World Health Organization; 2016
- [89] TB World Health Organization. Systematic Screening for Active Tuberculosis: Principles and Recommendations. Geneva: World Health Organization; 2013 (WHO/HTM/TB/2013.04; http://apps.who.int/iris/bitstream/10665/84971/1/9789241548601_eng.pdf?ua=1) [Accessed: 05 September 2017]
- [90] World Health Organization. Tuberculosis Prevalence Surveys: A Handbook. Geneva: World Health Organization; 2011 (WHO/HTM/TB/2010.17; http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/resources_documents/thelimebook/en/) [Accessed: 5 September 2017]