

Laboratory Diagnosis of Tuberculosis: State-of-the-art

Madhukar Pai MD PhD

Department of Epidemiology and Biostatistics, McGill International TB Center,
McGill University, Montreal, Canada

ABSTRACT

Early and accurate diagnosis is the first critical step in detecting and controlling tuberculosis (TB). All persons with cough lasting for 2 weeks or more, or with abnormal chest X-rays should be evaluated for TB. All persons with previous history of TB treatment, or with treatment failure must undergo drug-susceptibility testing (DST). For pulmonary TB, sputum (not blood) is the critical sample for laboratory testing. There are three accepted, validated sputum tests for microbiological confirmation of active TB: (i) sputum smear microscopy for acid-fast bacilli (AFB); (ii) nucleic acid amplification tests (NAATs), such as Xpert MTB/RIF[®] and line-probe assays (LPAs); and (iii) liquid cultures. For extrapulmonary TB (EPTB), samples from the site of the disease must be tested using Xpert, histopathology, and cultures. Chest X-rays are useful and sensitive but not specific for the diagnosis of pulmonary TB. Therefore, chest radiography needs to be followed by sputum testing for microbiological confirmation. If sputum smears and NAATs are negative, and TB is still suspected, liquid cultures are the most sensitive tests available for TB. Culture is, therefore, very useful in diagnosing smear-negative TB, pediatric TB, EPTB, as well as drug-resistant TB. Blood-based antibody tests [e.g., immunoglobulin G (IgG) or immunoglobulin M] (IgM), enzyme-linked immunosorbent assay (ELISA)], and interferon-gamma release assays are not recommended for active TB diagnosis. For rapid DST, LPAs and automated cartridge-based NAATs are recommended, followed by liquid culture and DST.

Email: madhukar.pai@mcgill.ca

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IMPORTANCE OF CASE FINDING

Despite the widespread implementation of the Stop TB Strategy, tuberculosis (TB) incidence continues to be high in many countries, with nearly 8.6 million cases and 1.3 million deaths occurring in 2012.¹ In the 2013 Global TB Report, World Health Organization (WHO) emphasized two major challenges for TB control: the “missing” 3 million people who develop active TB every year but never get reported to National TB Control Programs, and the crisis of multidrug-resistant TB (MDR-TB), in which three out of four people with MDR-TB are never diagnosed.¹ India alone accounts for 25% of the global incident cases, and accounts for nearly a third of the so-called “missing cases”.¹ These cases are presumably managed by India’s vast, unregulated private health sector.

By the time patients present to the health care system, get diagnosed, and receive anti-TB therapy; they may have already transmitted the infection to many others in their communities. Even when patients with chest symptoms present to the health care system, diagnostic delays are common because of inaccurate tests, delays in ordering and receiving test results, and inability of health systems to ensure rapid (e.g., same day) treatment. A recent systematic review has shown that an average TB patient in India is diagnosed after a delay of about 2 months, and is seen, on average, by nearly three health care providers before a diagnosis is made.² About half of all TB patients in India seek private or informal health care.³

Early and accurate diagnosis followed by rapid treatment, therefore, is critical to break the chain of transmission, especially in the absence of an effective vaccine. Kik and colleagues have proposed six strategies that may help optimize TB diagnosis to reduce transmission: (i) systematic screening to identify undiagnosed disease, especially among those not seeking care; (ii) facilitating earlier detection of TB by engaging first-contact (e.g., informal and private) providers; (iii) reducing misdiagnosis by eliminating suboptimal diagnostics (e.g., serological tests); (iv) improving diagnosis by scaling-up of WHO-endorsed diagnostics [e.g., Xpert MTB/RIF[®], line-probe assays (LPAs), and liquid cultures]; (v) implementing diagnostics as point-of-care testing programs to ensure rapid linkages to treatment; and (vi) using information and communication technologies (e.g., mobile phones) to ensure notification and treatment completion.⁴

New tools are critical for transformational progress in TB control. In diagnostics, much of the attention is focused on the global rollout of the Xpert MTB/RIF[®] technology with over 6 million cartridges used (by March 2014) in 95 of the 145 countries eligible for concessional pricing.⁵

STANDARDS FOR DIAGNOSIS

All patients with TB should receive the same quality of care, based on the best evidence available.⁶ The best practices for TB are enshrined in the International Standards of TB Care (ISTC), which was first published in 2006.⁷ The third edition of the ISTC (www.istcweb.org), published in March 2014, has put forth these standards for TB diagnosis:⁸

- *Standard 1:* to ensure early diagnosis, providers must be aware of individual and group risk factors for TB and perform prompt clinical evaluations and appropriate diagnostic testing for persons with symptoms and findings consistent with TB
- *Standard 2:* all patients (including children) with unexplained cough lasting for 2 or more weeks or with unexplained findings suggestive of TB on chest radiographs should be evaluated for TB
- *Standard 3:* all patients (including children) who are suspected of having pulmonary TB and are capable of producing sputum should have at least two sputum specimens submitted for smear microscopy or a single sputum specimen for Xpert MTB/RIF[®] testing in a quality-assured laboratory. Patients at risk for drug resistance, who have human immunodeficiency virus (HIV) risks, or who are seriously ill, should have Xpert MTB/RIF[®] performed as the initial diagnostic test. Blood-based serologic tests and interferon-gamma release assays (IGRAs) should not be used for diagnosis of active TB
- *Standard 4:* for all patients (including children) suspected of having extrapulmonary TB (EPTB), appropriate specimens from the suspected sites of involvement should be obtained for microbiological and histopathological examinations. An Xpert MTB/RIF[®] test is recommended as the preferred initial microbiological test for suspected tuberculous meningitis because of the need for a rapid diagnosis
- *Standard 5:* in patients suspected of having pulmonary TB whose sputum smears are negative, Xpert MTB/RIF[®] and/or sputum cultures should be performed. Among smear and Xpert MTB/RIF[®] negative persons with clinical evidence strongly suggestive of TB, anti-TB treatment should be initiated after collection of specimens for culture examination
- *Standard 6:* in all children suspected of having intrathoracic (i.e., pulmonary, pleural, and mediastinal or hilar lymph node) TB, bacteriological confirmation should be sought through examination of respiratory secretions (expectorated sputum, induced sputum, gastric lavage) for smear microscopy, an Xpert MTB/RIF[®] test, and/or culture
- *Standard 11:* an assessment of the likelihood of drug resistance, based on history of prior treatment, exposure to a possible source case having drug-resistant organisms, and the community prevalence of drug resistance (if known),

should be obtained for all patients. Drug-susceptibility testing (DST) should be performed at the start of therapy for all previously treated patients. Patients who remain sputum smear-positive at completion of 3 months of treatment and patients in whom treatment has failed, have been lost to follow-up, or relapsed following one or more courses of treatment should always be assessed for drug resistance. For patients in whom drug resistance is considered to be likely, an Xpert MTB/RIF[®] test should be the initial diagnostic test. LPA or culture and testing for susceptibility to at least isoniazid (INH) and RIF should be performed promptly if rifamycin resistance is detected.

DIAGNOSIS OF ACTIVE TUBERCULOSIS

Active TB, ideally, should be microbiologically confirmed. There are three methods that are recommended for microbiological confirmation of active TB disease: (i) smear microscopy; (ii) nucleic acid amplification tests (NAATs); and (iii) liquid cultures. Table 1 shows the widely accepted, WHO-endorsed technologies available for active TB diagnosis.⁹ These technologies are already under use in the Revised National TB Control Program (RNTCP) in India, and also available at affordable prices via the Initiative for Promoting Affordable and Quality TB Tests (IPAQT) in India (www.ipaqt.org).¹⁰

Optimized Smear Microscopy

Smear microscopy is a rapid, inexpensive, and widely available technology. While sensitivity is modest (50–70%), specificity is very high (>98%).¹¹ Based on several WHO policies,^{12,19} the following strategy is the most optimized form of smear microscopy: at least two sputum samples, stained with a fluorescence stain, and read by a well-trained microscopist using light-emitting diode (LED) microscopy, in a laboratory that is engaged in an ongoing external quality assurance (EQA) program.²⁰ LED microscopy (Figure 1) is WHO-endorsed, and already in use in several Medical Colleges in India. Recent research from India has clearly shown its impact in terms of increased sensitivity and case detection.²¹ Thus, where possible, traditional Ziehl-Neelsen microscopy should be upgraded to LED fluorescence microscopy.

Nucleic Acid Amplification Tests

In 2010, the WHO endorsed a new, rapid, automated, 2-hour molecular test called Xpert MTB/RIF (Figure 2), based on the GeneXpert[®] technology which can diagnose TB with high accuracy and can also detect those with RIF resistance (a very good marker of MDR-TB). A Cochrane review has shown that the



Figure 1: Light-emitting diode fluorescence microscopy for acid-fast bacilli.

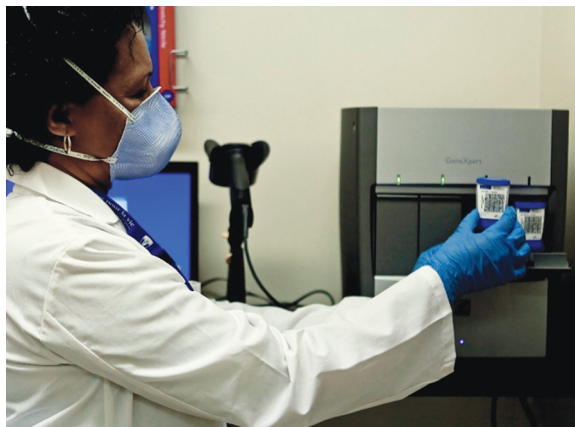


Figure 2: Xpert MTB/RIF[®] an automated 2-hour test for tuberculosis and rifampicin resistance.

Xpert MTB/RIF test has 88% sensitivity and 98% specificity when compared to culture.¹³ Even in smear-negative, culture-proven TB cases, Xpert has a sensitivity of about 68%. Xpert MTB/RIF can detect RIF resistance with a sensitivity of 95% and specificity of 98% (Table 1).¹³

Data from many countries, including India, clearly show substantially better performance of the Xpert MTB/RIF test over conventional smear microscopy. Newer data also suggest that Xpert MTB/RIF has value for EPTB, especially TB lymphadenitis and TB meningitis (reviewed elsewhere¹⁴).

Table 1: Validated, World Health Organization (WHO)-endorsed Tests for Active Tuberculosis and Drug-susceptibility Testing

Test type or platform (reference for evidence)	Test description	Current validated commercial versions	WHO endorsed? (reference to policy)	Implemented by RNTCP?	Goal of testing (to detect)	Sensitivity	Specificity
Smear microscopy ¹¹	LED fluorescence microscopy	Primo Star iLED™	Yes ¹²	Yes	Active TB	> 60% (compared to culture)	98% (compared to culture)
Automated, CB-NAAT ¹³	CB-NAAT is a self-contained and fully automated technological platform that integrates sputum processing, DNA extraction and amplification, TB and MDR-TB diagnosis	Xpert MTB/RIF®	Yes ¹⁴	Yes	Active TB and DST	For active TB: >98% in smear-positive patients and 68% in smear-negative For detecting drug resistance to RIF: 95%	For active TB: >98% For detecting drug-resistance to RIF: 98% >99%
Liquid TB culture ¹⁵	Fully automated system for mycobacterial liquid culture and DST	BACTEC MGIT® 960 BacT/ALERT® 3D	Yes ¹⁶	Yes	Active TB and DST	100% in smear-positive cases >75% in smear-negative cases	
LPAs ¹⁷	LPAs for mutations that confer resistance to INH and RIF	Hain Geno type MTBDR plus	Yes ¹⁸	Yes	Rapid DST for INH and RIF resistance	98% for RIF resistance and 84% for INH resistance	99% for RIF resistance and 99% specificity for INH

WHO, World Health Organization; RNTCP, Revised National TB Control Program; LED, light-emitting diode; TB, tuberculosis; CB-NAAT, cartridge-based nucleic acid amplification test; DNA, deoxyribonucleic acid; MDR-TB, multidrug-resistant TB; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; DST, drug-susceptibility testing; BD, Becton Dickinson; 3D, three dimensional; LPAs, line-probe assays INH, isoniazid.
Source: Modified from Pai M. Diagnosis of pulmonary tuberculosis: recent advances. *J Indian Med Assoc.* 2013;111:332-36.

In October 2013, WHO issued updated recommendations on the use of Xpert MTB/RIF, based on the recommendations of an Expert Group Meeting held in May 2013, which were subsequently endorsed by WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) in June 2013.¹⁴ The revised WHO recommendations on Xpert MTB/RIF for the diagnosis of pulmonary TB and RIF resistance in adults and children are:¹⁴

- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults presumed to have MDR-TB or HIV-associated TB (strong recommendation, high-quality evidence)
- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in children presumed to have MDR-TB or HIV-associated TB (strong recommendation, very low-quality evidence)
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all adults presumed to have TB (conditional recommendation acknowledging resource implications, high-quality evidence)
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all children presumed to have TB (conditional recommendation acknowledging resource implications, very low-quality evidence)
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults presumed to have TB but not at risk of MDR-TB or HIV-associated TB, especially in further testing of smear-negative specimens (conditional recommendation acknowledging resource implications, high-quality evidence).

The revised recommendations on EPTB are:²²

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing cerebrospinal fluid (CSF) specimens from patients presumed to have TB meningitis (strong recommendation given the urgency of rapid diagnosis, very low-quality evidence)
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific nonrespiratory specimens (lymph nodes and other tissues) from patients presumed to have EPTB (conditional recommendation, very low-quality evidence).

Evidence from systematic reviews that informed the revised recommendations are included in the Report of the Expert Group Meeting and the policy itself.¹⁴ These revised recommendations have been incorporated into the third edition of the ISTC.

In 2013, UNITAID and WHO started the largest rollout of Xpert MTB/RIF. UNITAID has invested US\$ 25.9 million to purchase over 220 GeneXpert[®] machines and 1.4 million test cartridges for 21 countries in Africa, Eastern Europe, and Asia. Coordinated by the WHO and the Stop TB Partnership, this TB Xpert project is estimated to save an additional 62,000 lives. In India, the RNTCP has begun using Xpert MTB/RIF to rapidly expand access to DST in the program, with linkages to Programmatic Management of Drug-resistance (PMDT).

In 2008, the WHO endorsed the use of LPAs for rapid detection of resistance to INH and RIF resistance.¹⁸ These assays have high accuracy for RIF resistance and moderately high accuracy for INH resistance (Table 1).¹⁷

Liquid Cultures

Liquid cultures are the gold standard for active TB diagnosis (Table 1).^{15,23} Commercial systems like BACTEC MGIT[®] 960 and BacT/ALERT[®] 3D are WHO-endorsed and can provide results within 10–14 days, and MGIT can provide phenotypic DST results for first-line and select second-line drugs.¹⁶ Reliability of second-line DST is limited and, therefore, results should be carefully correlated with history of TB drugs taken in the past. Liquid culture and DST are key parts of any diagnostic algorithm, and should be routinely used in all patients with history of previous TB treatment or treatment failure. Liquid culture is also helpful in paucibacillary TB cases (EPTB, smear-negative TB, and childhood TB).

Role of Blood-based, Immunological Tests

Although several blood-based tests are commercially available for TB, none of these are accurate.²⁴ Antibody-based tests [immunoglobulin G (IgG), immunoglobulin M (IgM), and enzyme-linked immunosorbent assay (ELISA)] are inaccurate and produce highly inconsistent results.²⁵ WHO issued a strong, negative recommendation against their use in 2011, and this is also reflected in the ISTC.²⁶ Based on the WHO recommendations, the Indian Government banned serological antibody tests in 2012 and issued public notices about the ban.

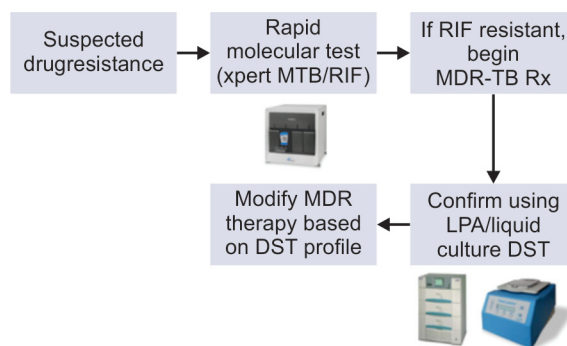
Other blood tests that are commercially available are called IGRAs. These assays (e.g., TB Gold; TB Platinum) are intended to diagnose latent TB infection (LTBI) and are based on T-cell mediated, cellular immune response. These tests are intended to replace the Mantoux tuberculin skin test (TST). But neither TST nor IGRAs can separate latent infection from active TB disease,²⁷ and both tests are not recommended for active TB diagnosis by WHO, ISTC, and RNTCP.²⁸ If IGRAs are used for active TB diagnosis, they can result in substantial overtreatment of TB, since nearly 40% of the Indian adult population is estimated to be latently infected.²⁹

Diagnosis of Extrapulmonary Tuberculosis

For EPTB, it is critical that physicians collect samples from the site of the disease. These include tissue (biopsy), fluids (e.g., CSF, pleural fluid), gastric juice, and other aspirates. No single test performs well in EPTB samples because of the low bacillary load. Multiple tests, therefore, need to be used, including Xpert MTB/RIF, histopathology, and cultures. WHO has recently endorsed the use of Xpert for TB meningitis, lymphadenitis, and pediatric TB (gastric aspirates).¹⁴ Xpert MTB/RIF, however, does not have high sensitivity for pleural samples.¹⁴ Thus, pleural biopsy and cultures are continue to be important tools for TB pleuritis.³⁰

DIAGNOSIS OF DRUG-RESISTANT TUBERCULOSIS

According to ISTC, DST should be performed at the start of therapy for all previously treated patients. In addition, DST must be performed on all patients who remain sputum smear-positive at completion of 3 months of treatment and patients in whom treatment has failed, have been lost to follow-up, or relapsed. For DST, both WHO and ISTC now recommend Xpert MTB/RIF[®] test as the initial diagnostic test because it can rapidly detect RIF resistance within 90 minutes with high accuracy, and allow clinicians to initiate empiric MDR therapy, pending confirmation.¹⁴ LPAs (e.g., Hain GenoType[®] MTBDRplus) or liquid culture and DST should then be performed promptly if RIF resistance is detected using Xpert MTB/RIF. This algorithm is shown in figure 3. Once culture and DST results are obtained, MDR therapy can be customized to the patient's drug-susceptibility profile, and must include a combination of at least five drugs to which the TB bacilli are still sensitive.



MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; MDR-TB, multidrug-resistant tuberculosis; DST, drug-susceptibility testing; LPAs, line-probe assays.

Figure 3: Algorithm for rapid drug-susceptibility testing, followed by confirmation.

DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION

Most individuals contain MTB infection using host defense mechanisms, and the infection remains “latent”. LTBI can progress to active TB disease in about 10% of those with infection, over a lifetime. INH preventive therapy (also called prophylactic treatment) can reduce the risk of progression to active disease by about 60%.³¹ Thus, detection of LTBI, followed by INH preventive therapy, is a useful strategy in specific high-risk groups. These include young children (under 5 years of age) who are in close contact with an active TB case; individuals with HIV-infection; individuals with immunocompromising conditions, such as cancer, diabetes, renal failure, and those on immunosuppressive medications, such as tumor necrosis factor-alpha (TNF- α) blockers.

However, there is no diagnostic gold standard for LTBI. Two tests are available currently: TST (performed using Mantoux technique) and IGRAs. Evidence suggests that both TST and IGRAs are acceptable but imperfect tests.³² They represent indirect markers of MTB exposure and indicate a cellular immune response to MTB. Neither tests can accurately differentiate between LTBI and active TB, distinguish reactivation from reinfection or resolve the various stages within the spectrum of MTB infection.³² In addition, both have reduced sensitivity in immunocompromised patients (e.g., HIV-infected).³³ Both tests have low predictive value for progression to active TB.³⁴ To maximize the positive predictive value of existing tests, LTBI screening should be reserved only for those who are at sufficiently high risk of progressing to disease to outweigh the known harms of LTBI treatment. Most importantly, both TST and IGRAs should not be used for the diagnosis of active TB in high endemic settings like India. In children, these tests may have some value as a test for infection, in addition to chest X-rays, symptoms, history of contact, and other microbiological investigations (e.g., gastric juice AFB and Xpert MTB/RIF).²²

NOTIFICATION OF TUBERCULOSIS CASES

As per Government of India order published in May 2012, all TB cases must be notified to Nodal Public Health Authorities (e.g., District or Municipal Health Officers), including cases managed in the private and non-governmental organization (NGO) sectors. For notification, a microbiologically-confirmed TB case is a patient diagnosed with at least one sputum specimen positive for AFB, or culture-positive for MTB, or RNTCP-approved rapid molecular test positive for TB. A clinical TB case is a patient diagnosed clinically as TB, without microbiologic confirmation and initiated on anti-TB drugs. The RNTCP has set up a special web portal for notification (nikshay.gov.in) and has also published a guidance tool for notification (www.tbcindia.nic.in/).

CONCLUSION

All health care providers need to actively investigate for TB in everyone with 2 weeks of cough or abnormal chest X-rays. WHO-endorsed sputum tests (smears, Xpert MTB/RIF, LPA, and liquid cultures) should be used to diagnose TB and MDR-TB, and all cases must be notified to local Public Health Authorities. Blood tests (antibody tests and IGRAs, like TB Gold and TB Platinum) are not recommended for active TB. For EPTB, samples from the site of the disease must be tested using Xpert, histopathology, and cultures. Ideally, all TB patients should undergo DST. At the very least, everyone with previous history of TB (or not responding to standard therapy) must undergo DST for MDR-TB. Lastly, tests that are intended for LTBI (e.g., Mantoux and TB Gold) must be restricted for latent TB diagnosis, and not abused for active disease detection.

Editor's Comment

It is very important and crucial to have an early and accurate in detecting and controlling TB. A TB suspect is one who has cough for more than 2 weeks and all such persons are to be tested for sputum AFB and if negative a chest X-ray. All persons with cough lasting for 2 weeks or more, or with abnormal chest X-rays should be evaluated for TB. All persons with previous history of TB treatment, or with treatment failure must undergo DST. For pulmonary TB, sputum (not blood) is the critical sample for laboratory testing. There are three accepted, validated sputum tests for microbiological confirmation of active TB: (i) sputum smear microscopy for AFB; (ii) NAATs, such as Xpert MTB/RIF and LPAs; and (iii) liquid cultures. For EPTB, samples from the site of the disease must be tested using Xpert, histopathology, and cultures. Chest X-rays are useful and sensitive but not specific for the diagnosis of pulmonary TB. Therefore, chest radiography needs to be followed by sputum testing for microbiological confirmation. If sputum smears and NAATs are negative, and TB is still suspected, liquid cultures are the most sensitive tests available for TB. Culture is, therefore, very useful in diagnosing smear-negative TB, pediatric TB, EPTB, as well as drug-resistant TB. Blood-based antibody tests (e.g., IgG or IgM, ELISA) and IGRAs are not recommended for active TB diagnosis. For rapid DST, LPAs, and automated CB-NAATs are recommended, followed by liquid culture and DST.

Digambar Behera

REFERENCES

1. World Health Organization. (2013). Global Tuberculosis Report 2013. [online] Available from: apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf. [Accessed April, 2014].
2. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. *Int J Tuberc Lung Dis*. 2014;18:255-66.
3. Satyanarayana S, Nair SA, Chadha SS, Shivashankar R, Sharma G, Yadav S, et al. From where are tuberculosis patients accessing treatment in India? Results from a cross-sectional community based survey of 30 districts. *PLoS One*. 2011;6:e24160.
4. Kik SV, Qin ZZ, Pai M. (2014). Optimal diagnosis: how early and improved diagnosis can help prevent TB transmission. [online] Available from: www.futuremedicine.com/doi/abs/10.2217/ebo.13.644. [Accessed April, 2014].
5. World Health Organization. (2013). WHO monitoring of Xpert MTB/RIF roll-out. [online] Available from: www.who.int/tb/laboratory/mtbrifrollout/en/index.html. [Accessed April, 2014].
6. Pai M, Das J. Management of tuberculosis in India: time for a deeper drive into quality. *Natl Med J India*. 2013;26:65-8.
7. Hopewell PC, Pai M, Maher D, Uplekar M, Raviglione MC. International standards for tuberculosis care. *Lancet Infect Dis*. 2006;6:710-25.
8. TB CARE I. International Standards for Tuberculosis Care, 3rd Edition. Available from: www.istcweb.org 2014. Accessed July 2014.
9. Pai M. Diagnosis of pulmonary tuberculosis: recent advances. *J Indian Med Assoc*. 2013;111:332-36.
10. Pai M. Promoting affordable and quality tuberculosis testing in India. *J Lab Physicians*. 2013;5:1-4.
11. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6:570-81.
12. World Health Organization. (2011). Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: policy statement. [online] Available from: whqlibdoc.who.int/publications/2011/9789241501613_eng.pdf. [Accessed April, 2014].
13. Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev*. 2013;1:CD009593.
14. World Health Organization. (2013). Policy update: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children. [online] Available from: http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf. [Accessed July 2014].
15. Cruciani M, Scarpato 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. *J Clin Microbiol*. 2004;42:2321-5.
16. World Health Organization. (2007). The use of liquid medium for culture and DST. [online] Available from: www.who.int/tb/laboratory/policy_liquid_medium_for_culture_dst/en/index.html. [Accessed April, 2014].
17. Ling DI, Zwerling A, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *Eur Respir J*. 2008;32:1165-74.
18. World Health Organization. (2011). Policy statement: Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). [online] Available from: www.who.int/tb/laboratory/policy_statements/en/index.html. [Accessed April, 2014].
19. World Health Organization. (2011). Same-day diagnosis of tuberculosis by microscopy: policy statement. [online] Available from: whqlibdoc.who.int/publications/2011/9789241501606_eng.pdf. [Accessed April, 2014].
20. Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Rev Anti Infect Ther*. 2007;5:327-31.
21. Reza LW, Satyanarayana S, Enarson DA, Kumar AM, Sagili K, Kumar S, et al. LED-fluorescence microscopy for diagnosis of pulmonary tuberculosis under programmatic conditions in India. *PLoS One*. 2013;8:e75566.

22. Mandalakas AM, Detjen AK, Hesselning AC, Benedetti A, Menzies D. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis*. 2011;15:1018-32.
23. Horne DJ, Pinto LM, Arentz M, Lin SY, Desmond E, Flores LL, et al. Diagnostic accuracy and reproducibility of WHO-endorsed phenotypic drug susceptibility testing methods for first-line and second-line antituberculosis drugs. *J Clin Microbiol*. 2013;51:393-401.
24. Grenier J, Pinto L, Nair D, Steingart K, Dowdy D, Ramsay A, et al. Widespread use of serological tests for tuberculosis: data from 22 high-burden countries. *Eur Respir J*. 2012;39:502-5.
25. Steingart KR, Flores LL, Dendukuri N, Schiller I, Laal S, Ramsay A, et al. Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and meta-analysis. *PLoS Med*. 2011;8:e1001062.
26. World Health Organization. (2011). Policy statement: Commercial serodiagnostic tests for diagnosis of tuberculosis. [online] Available from: www.who.int/tb/laboratory/roadmap_xpert_mtb_rif_rev23dec2010.pdf. [Accessed April, 2014].
27. Metcalfe JZ, Everett CK, Steingart KR, Cattamanchi A, Huang L, Hopewell PC, et al. Interferon- γ release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis. *J Infect Dis*. 2011;204:S1120-9.
28. World Health Organization. (2011). Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries: Policy statement. [online] Available from: whqlibdoc.who.int/publications/2011/9789241502672_eng.pdf. [Accessed April, 2014].
29. Chadha VK. Tuberculosis epidemiology in India: a review. *Int J Tuberc Lung Dis*. 2005;9:1072-82.
30. Trajman A, Pai M, Dheda K, van Zyl Smit R, Zwerling AA, Joshi R, et al. Novel tests for diagnosing tuberculous pleural effusion: what works and what does not? *Eur Respir J*. 2008;31:1098-106.
31. Landry J, Menzies D. Preventive chemotherapy. Where has it got us? Where to go next? *Int J Tuberc Lung Dis*. 2008;12:1352-64.
32. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Interferon-gamma release assays for the detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev*. 2014;27:3-20.
33. Cattamanchi A, Smith R, Steingart KR, Metcalfe JZ, Date A, Coleman C, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. *J Acquir Immune Defic Syndr*. 2011;56:230-8.
34. Rangaka MX, Wilkinson KA, Glynn JR, Ling D, Menzies D, Mwansa-Kambafwile J, et al. Predictive value of interferon- γ release assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2012;12:45-55.