Novel and Improved Technologies for Tuberculosis Diagnosis: Progress and Challenges

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KEYWORDS

• Tuberculosis • Diagnostics • New tools
• Sensitivity and specificity

Despite substantial success in implementing standardized care and improving rates of cure in recent years, the global burden of tuberculosis (TB) remains enormous. Lack of rapid and accurate diagnosis and case detection are major obstacles to TB control. TB diagnosis, even today, continues to rely heavily on tools such as direct smear microscopy, solid culture, chest radiography, and tuberculin skin testing: tools that often perform poorly, and require infrastructure frequently unavailable in the periphery of the health system where patients first seek care. The limitations of the existing diagnostics toolbox have been exposed by the human immunodeficiency virus (HIV) epidemic[1,2] and by the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). Diagnostic delays and health system failures often result in missed or late diagnoses, with serious consequences for TB patients.[3]

In the past few years, there has been an unprecedented level of interest and activity focused on the development of new tools for TB diagnosis, largely because of agencies such as the Foundation for Innovative New Diagnostics (FIND), the Stop TB Partnership's New Diagnostics Working Group (NDWG), the Global Laboratory Initiative (GLI) (another Stop TB Partnership Working Group), the World Health Organization (WHO), and the Special Program for Research and Training in Tropical Diseases (TDR).[2,4–6] Funding agencies such as the Bill & Melinda Gates Foundation, the Global Fund to Fight AIDS, TB and Malaria (GFATM), and UNITAID have provided the much-needed resources and impetus to push the new tools agenda, in keeping with the Global Plan to Stop TB.[7]

This article reviews the existing evidence base of TB diagnostics, describes new technologies and the progress made in their development and

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evaluation, and ends with a review of cost-effectiveness and modeling studies of the potential impact of new diagnostics in TB control.

THE EVIDENCE BASE OF TB DIAGNOSIS

Although primary diagnostic trials are performed to generate data on test accuracy and operational performance, systematic reviews and meta-analyses provide the best synthesis of current evidence on any given diagnostic test. In the past few years, more than 30 systematic reviews and meta-analyses have been published on various TB tests.[8] These reviews have synthesized the results of more than 1000 primary studies and have provided useful insights into the diagnostic accuracy and role of various tests (**Table 1**).[8] They have also played a key role in recent policy statements and guidelines on TB diagnostics.[9] However, much of the existing evidence base is focused on test accuracy (ie, sensitivity and specificity). There are limited data on outcomes such as accuracy of diagnostic algorithms (rather than single tests) and their relative contributions to the health care system, incremental value of new tests, effect of new tests on clinical decision-making and therapeutic choices, cost-effectiveness in routine programmatic settings, and effect on patient-centered outcomes.[8] Recently, NDWG launched a comprehensive Web site resource ''Evidence-based tuberculosis diagnosis,'' available at http://www.tbevidence.org/ (**Fig. 1**). This Web site is the most comprehensive single source of evidence syntheses, policies, guidelines, and research agendas on TB diagnosis.

IMPROVED AND NEW TECHNOLOGIES: WHAT'S IN THE PIPELINE?

New diagnostics pipeline for TB is rapidly expanding. In 2008, the Stop TB Partnership's Retooling Task Force (RTF) and NDWG produced a detailed brochure on diagnostic tools in the pipeline, mainly to provide guidance to National TB Programs (NTPs), and for funding and technical agencies that may wish to support the development, evaluation, or implementation of new tools.[10] **Fig. 2** shows the pipeline; the tools are stratified as ''WHO-endorsed,'' ''Tools in late-stage development/evaluation,'' or ''Tools in early phase development.'' The figure not only describes the various tests but also provides some information on the commercial kits available, training requirements, and estimated costs.[10] A more exhaustive list of various TB technologies was published by Perkins and Cunningham.[1] Some of the technologies are described in greater detail in subsequent sections.

OPTIMIZED SMEAR MICROSCOPY

Although much work is being done to develop new diagnostics, in most resource-limited countries direct sputum smear microscopy remains the primary means for diagnosis of TB. Given the known limitations of smear microscopy, considerable effort has been given to identifying methods that can optimize the yield and accuracy of smear microscopy.[11–14] These include light-emitting diode (LED)-based fluorescence microscopy (FM), use of sputum processing methods, and optimization of specimen collection for same-day diagnosis[15] (ie, front-loaded microscopy). **Fig. 3** provides an overview of the major commercial LED technologies for microscopy.[16] Although published data are limited (reviewed by Minion and colleagues[16]), LED technologies seem to be promising in settings in which FM has not been feasible, and a WHO policy on LED microscopy is expected in November 2009. Mobile phone-based microscopy[17] and automated detection systems using image processing[18] are other novel approaches that have been proposed, although the use of these approaches is yet to be adequately validated.

IMPROVED AND NEWER CULTURE METHODS

Automated Liquid Cultures

Automated liquid culture systems such as BacT/ALERT MP (bioMerieux Inc, Durham, NC, USA) and BD BACTEC MGIT (Becton Dickinson, Sparks, MD, USA) are currently considered the gold-standard approach for isolating mycobacteria. Meta-analyses have shown that liquid systems are more sensitive for detection of mycobacteria and may increase the case yield by 10% compared with solid media.[19,20] They also reduce the delays in obtaining results to days rather than weeks. Use of liquid media for drug susceptibility results in even greater time savings. However, liquid systems are prone to contamination and require stringent quality assurance systems and training standards. In addition, they are more expensive and require equipment investments, though MGIT can also be used as a manual system. Traditionally, liquid culture has always been used in tandem with solid media to maximize yield and allow examination of colony morphology. FIND projects demonstrated the feasibility of using liquid culture as a stand-alone method if rapid species confirmation is possible through the use of rapid antigen detection tests for speciation.

There are currently 3 manufacturers of these rapid tests, which detect the TB-specific protein MPT64 in a lateral flow format (eg, Capilia-TB, TAUNS, Numazu, Japan).[21] In 2007, WHO released a policy statement on the use of liquid culture systems and on species confirmation through antigen detection.[22] The WHO policy recommends phased implementation of these systems as a part of a country-specific comprehensive plan for laboratory capacity strengthening, and addresses key issues, including biosafety, customer support, staff training, maintenance of infrastructure and equipment, specimen transport, and reporting of results.[22]

Unconventional and Newer Culture Methods

Because commercial automated liquid cultures are expensive and may require sophisticated instrumentation, several researchers have proposed unconventional and novel culture-based approaches for TB diagnosis and drug resistance testing. These approaches include microscopic observation drug-susceptibility test (MODS),[23] thin-layer agar (TLA),[24] and the direct nitrate reductase assay (NRA),[25] also known as the Griess method. Recent reviews have summarized their characteristics and potential role.[26–28] Although these methods are promising as they allow the use of inexpensive materials and give turnaround times similar to liquid culture, these tests are not well standardized, and require extensive training and optimization before routine clinical use. These methods all require routine specimen processing, the most burdensome component of mycobacterial culture, before direct inoculation with sputum.

As for all culture-based methods, quality assurance is critical to minimize contamination and to ensure biosafety standards are followed. Appropriate quality-control systems are often lacking, recommended equipment (such as biosafety cabinets) may be unavailable, and strict adherence to infection control practices is infrequently enforced in resource-limited settings. Some of these novel culture-based assays have attempted to address laboratory safety issues inherent in the culturing of *Mycobacterium tuberculosis* by sealing the inoculated cultures in transparent plates or tubes, and relying on visual inspection of typical colony morphology (MODS and TLA) or color changes (Griess) to identify TB growth. Although disposal of the biohazardous material remains a concern, minimizing the need for direct handling and manipulation of mycobacterial cultures by laboratory technologists is an important advantage.

MOLECULAR TESTS

Nucleic acid amplification tests (NAATs) have been in use for many years, although their use has been largely restricted to high-income countries. For example, the 2009 updated guideline on use of NAATs by the US Centers for Disease Control and Prevention (CDC) states that ''NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities, such as contact investigations.''[29] Clearly, this recommendation is focused on high-income settings that have the resources to implement these guidelines.

As demonstrated in several meta-analyses, existing NAATs have high specificity, but modest and variable sensitivity, especially in smear-negative and extrapulmonary TB.[30–33] Several newer NAATs have been developed recently, including 2 technologies codeveloped with FIND, the loop-mediated isothermal amplification (Eiken Chemical Co Ltd, Tokyo, Japan), a simplified manual NAAT designed for peripheral laboratory facilities,[34] and the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), a fully automated NAAT platform that can detect TB and rifampin resistance.[35] Both of these tests are formatted for use outside reference centers, to replace or supplement microscopy at health centers and district hospitals. These tests have shown great promise in early studies, although published evidence is still limited. FIND is currently evaluating these tests in high-burden countries. The Xpert MTB/RIF assay has recently been CE marked with package insert data showing greater than 95% detection of all TB patients.

Line probe assays (LPAs) have recently been introduced in many countries for molecular detection of drug resistance from smear-positive specimens. Two commercial LPAs are available: the INNO-LiPA Rif.TB (Innogenetics NV, Gent, Belgium) and GenoType MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany). Meta-analyses have shown that LPAs are highly accurate, and the GenoType assay, in particular, performs well for rapid detection of rifampin resistance in smear-positive sputum specimens.[36,37] In 2009, a newer assay (GenoType MTBDR*sl* assay) became available.[38] This assay allows the simultaneous detection of the *M tuberculosis* complex and resistance to fluoroquinolones or aminoglycosides/cyclic peptides or ethambutol from smear-positive pulmonary specimens or culture isolates. Thus, the combined use of GenoType MTBDR*plus* and

Table 1
Summary of findings from several systematic reviews onTB diagnostic tests

Diagnostic Test	Disease/Site	Major Findings/Results of Systematic Reviews
Diagnosis of active TB		
Sputum smear microscopy	Pulmonary TB	■ FM is on average 10% more sensitive than conventional microscopy. Specificity of FM and conventional microscopy is similar. FM is associated with improved time efficiency ■ Centrifugation and overnight sedimentation preceded by any of several chemical methods (including bleach) are more sensitive than direct microscopy; specificity is unaffected by sputum-processing methods ■ When serial sputum specimens are examined, the mean incremental yield or increase in sensitivity from examination of third sputum specimen ranges between 2% and 5%
NAATs	Pulmonary and extrapulmonary TB	NAATs have high specificity and positive predictive value. However, they have lower (and highly variable) sensitivity and negative predictive value for all forms of TB, especially in smear-negative and extrapulmonary disease. In-house ("home brew") NAATs produce highly inconsistent results compared with commercial, standardized NAATs
Commercial serologic antibody detection tests	Pulmonary and extrapulmonary TB	Serologic tests for pulmonary and extrapulmonary TB produce inconsistent estimates of sensitivity and specificity; none of the assays performs well enough to replace microscopy
ADA	TB pleuritis, pericarditis, peritonitis	Measurement of ADA levels in pleural, pericardial, and ascitic fluid has high sensitivity and specificity for extrapulmonary TB
IFN-γ	TB pleuritis	Pleural fluid IFN-γ determination is a sensitive and specific test for the diagnosis of TB pleuritis
Phage amplification assays	Pulmonary TB	Phage-based assays have high specificity but lower and variable sensitivity. Their performance characteristics are similar to sputum microscopy
Automated liquid cultures	Pulmonary TB	Automated liquid cultures are more sensitive than solid cultures; time to detection is more rapid than solid cultures

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Table 1
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Diagnostic Test	Disease/Site	Major Findings/Results of Systematic Reviews
Diagnosis of latent TB		
TST	Latent TB infection	▪ Individuals who have received BCG vaccination are more likely to have a positive TST; the effect of BCG on TST results is less after 15 years; positive TST with indurations of greater than 15 mm are more likely to be the result of TB infection than of BCG vaccination ▪ The effect on TST of BCG received in infancy is minimal, especially 10 years after vaccination. BCG received after infancy produces more frequent, more persistent, and larger TST reactions. NTM infection is not a clinically important cause of false-positive TST, except in populations with a high prevalence of NTM sensitization and a low prevalence of TB infection
T-cell–based IGRAs	Latent TB infection	IGRAs have excellent specificity (higher than the TST), and are unaffected by prior BCG vaccination
Diagnosis of drug resistance		
Phage amplification assays	Rapid detection of rifampicin resistance	When used on culture isolates, phage assays have high sensitivity, but variable and lower specificity. In contrast, evidence is lacking about the accuracy of these assays when they are directly applied to sputum specimens
LPAs: INNO-LiPA Rif.TB (LiPA) and GenoType MTBDR assays	Rapid detection of rifampicin resistance	LiPA is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test has lower sensitivity when used directly on clinical specimens. The GenoType MTBDR assays have excellent sensitivity and specificity for rifampicin resistance even when directly used on clinical specimens
Colorimetric redox-indicator methods and NRAs	Rapid detection of rifampicin and isoniazid resistance	Colorimetric methods and NRAs are highly sensitive and specific for the rapid detection of rifampicin and isoniazid resistance in culture isolates

Abbreviations: ADA, adenosine deaminase; NTM, nontuberculous mycobacterial.
Adapted from Pai M, Ramsay A, O'Brien R. Evidence-based tuberculosis diagnosis. PLoS Med 2008;5(7):e156.

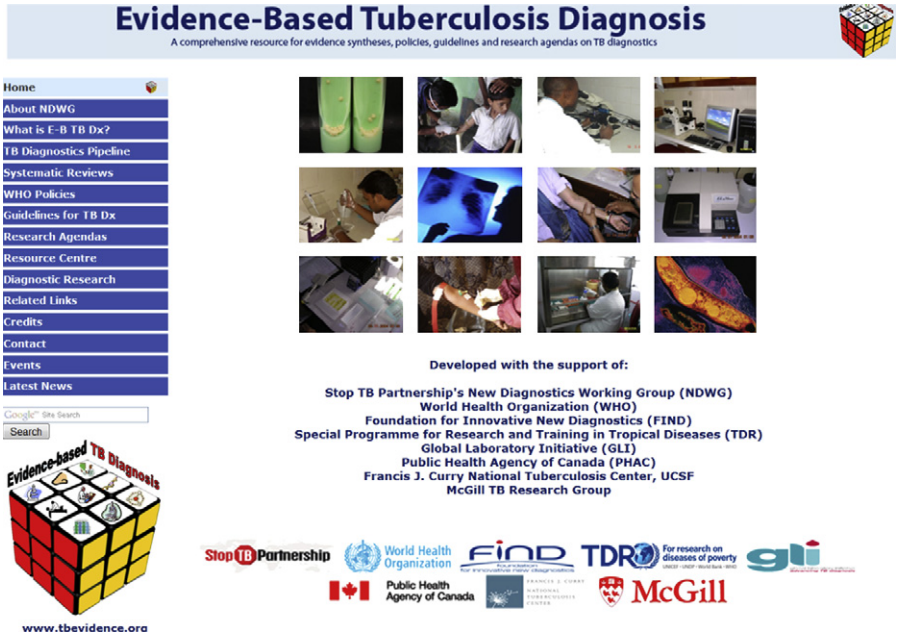
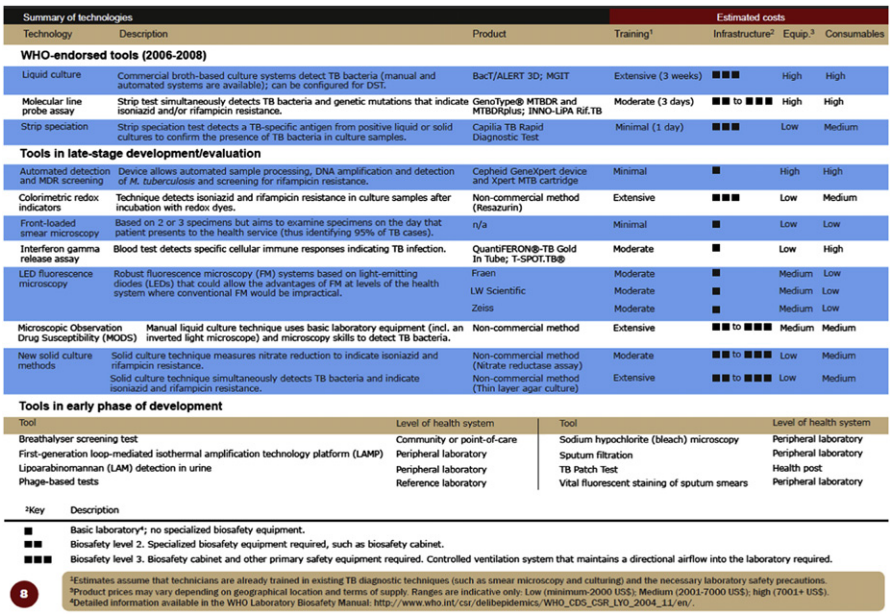


Fig. 1. Home page of the Web site "Evidence-based TB Diagnosis," http://www.tbevidence.org. (*Courtesy of* the Stop TB Partnership's New Diagnostics Working Group; with permission. Available at: http://www.tbevidence.org.)

GenoType MTBDRs*l* allows the rapid detection of XDR-TB. LPAs currently require routine specimen processing, DNA extraction, and conventional polymerase chain reaction analysis in a multiroom facility, and are thus limited to use in reference laboratories.

In 2008, WHO endorsed the use of LPAs for rapid detection of MDR-TB at the country level.[39] In 2009, UNITAID approved funding for a program called EXPAND-TB that will supply MDR-TB diagnostics to high-burden countries.[40] With a new grant of US$61,482,085, the project, led



Summary of technologies			Estimated costs			
Technology	Description	Product	Training[1]	Infrastructure[2]	Equip.[3]	Consumables
WHO-endorsed tools (2006-2008)						
Liquid culture	Commercial broth-based culture systems detect TB bacteria (manual and automated systems are available); can be configured for DST.	BacT/ALERT 3D; MGIT	Extensive (3 weeks)	■■■	High	High
Molecular line probe assay	Strip test simultaneously detects TB bacteria and genetic mutations that indicate isoniazid and/or rifampicin resistance.	GenoType® MTBDR and MTBDRplus; INNO-LIPA Rif.TB	Moderate (3 days)	■■ to ■■■	High	High
Strip speciation	Strip speciation test detects a TB-specific antigen from positive liquid or solid cultures to confirm the presence of TB bacteria in culture samples.	Capilia TB Rapid Diagnostic Test	Minimal (1 day)	■■■	Low	Medium
Tools in late-stage development/evaluation						
Automated detection and MDR screening	Device allows automated sample processing, DNA amplification and detection of *M. tuberculosis* and screening for rifampicin resistance.	Cepheid GeneXpert device and Xpert MTB cartridge	Minimal	■	High	High
Colorimetric redox indicators	Technique detects isoniazid and rifampicin resistance in culture samples after incubation with redox dyes.	Non-commercial method (Resazurin)	Extensive	■■■	Low	Medium
Front-loaded smear microscopy	Based on 2 or 3 specimens but aims to examine specimens on the day that patient presents to the health service (thus identifying 95% of TB cases).	n/a	Minimal	■	Low	Low
Interferon gamma release assay	Blood test detects specific cellular immune responses indicating TB infection.	QuantiFERON®-TB Gold In Tube; T-SPOT.TB®	Moderate	■	Low	High
LED fluorescence microscopy	Robust fluorescence microscopy (FM) systems based on light-emitting diodes (LEDs) that could allow the advantages of FM at levels of the health system where conventional FM would be impractical.	Fraen	Moderate	■	Medium	Low
		LW Scientific	Moderate	■	Medium	Low
		Zeiss	Moderate	■	Medium	Low
Microscopic Observation Drug Susceptibility (MODS)	Manual liquid culture technique uses basic laboratory equipment (incl. an inverted light microscope) and microscopy skills to detect TB bacteria.	Non-commercial method	Extensive	■■ to ■■■	Medium	Medium
New solid culture methods	Solid culture technique measures nitrate reduction to indicate isoniazid and rifampicin resistance.	Non-commercial method (Nitrate reductase assay)	Moderate	■■ to ■■■	Low	Medium
	Solid culture technique simultaneously detects TB bacteria and indicate isoniazid and rifampicin resistance.	Non-commercial method (Thin layer agar culture)	Extensive	■■ to ■■■	Low	Medium

Tools in early phase of development

Tool	Level of health system	Tool	Level of health system
Breathalyser screening test	Community or point-of-care	Sodium hypochlorite (bleach) microscopy	Peripheral laboratory
First-generation loop-mediated isothermal amplification technology platform (LAMP)	Peripheral laboratory	Sputum filtration	Peripheral laboratory
Lipoarabinomannan (LAM) detection in urine	Peripheral laboratory	TB Patch Test	Health post
Phage-based tests	Reference laboratory	Vital fluorescent staining of sputum smears	Peripheral laboratory

[2]Key Description

■ Basic laboratory[4]; no specialized biosafety equipment.
■■ Biosafety level 2. Specialized biosafety equipment required, such as biosafety cabinet.
■■■ Biosafety level 3. Biosafety cabinet and other primary safety equipment required. Controlled ventilation system that maintains a directional airflow into the laboratory required.

[1]Estimates assume that technicians are already trained in existing TB diagnostic techniques (such as smear microscopy and culturing) and the necessary laboratory safety precautions.
[3]Product prices may vary depending on geographical location and terms of supply. Ranges are indicative only: Low (minimum-2000 US$); Medium (2001-7000 US$); high (7001+ US$).
[4]Detailed information available in the WHO Laboratory Biosafety Manual: http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/.

8

Fig. 2. Summary of new technologies by the RTF and NDWG. (*From* World Health Organization & Stop TB Partnership. New laboratory diagnostic tools for tuberculosis control. Geneva: World Health Organization; 2008; with permission.)

Commercial LED products currently available for TB diagnostics

	Primo Star iLED	Lumin	ParaLens	FluoLED	CyScope
					
Manufacturer	Carl Zeiss Oberkochen, Germany	LW Scientific Lawrenceville, GA, USA	QBC Diagnostics Philipsburg, PA, USA	Fraen Settimo Milanese, Italy	Partec Gorlitz, Germany
Stand-alone microscope	Yes	No	No	No	Yes
Attachment	NA	Objective Lens Replacement (20X, 40X, 60X, 100X oil)	Objective Lens Replacement (40X, 60X oil, 100X oil)	Adaptor Attached to Base and Filter Installed on Head of Microscope	NA
Light Transmission	Epifluorescent	Epifluorescent	Epifluorescent	Transfluorescent	Epifluorescent
Battery Power	Yes	Yes	Yes	Yes	Yes
Weight	9.5kg	448g	1.27kg	5kg	2.7kg

Fig. 3. Commercial LED products currently available for TB diagnostics. (*Adapted from* Minion J, Sohn H, Pai M. Light emitting diode technologies for TB diagnosis: what's on the market? Expert Rev Med Devices 2009;6(4):341–45; with permission. Images have been reproduced with permission from the respective companies.)

by the GLI in close collaboration with FIND and the Global Drug Facility, will expand the use of LPAs for rapid MDR-TB diagnosis.[40] A key component of this initiative will be the strengthening of laboratories in countries where LPAs will be introduced in a phased manner, through collaboration between various partners. Strengthening of laboratory capacity is critical for the success of this program, and indeed, for the successful implementation of any new TB technology.

IMMUNE-BASED TESTS

Serologic, Antibody Detection Tests

Systematic reviews have reported strong evidence that existing commercial serologic tests are of little clinical value because of suboptimal accuracy and high inconsistent results.[41,42] This was reaffirmed in a recent study of 19 commercial tests by TDR/WHO, which showed suboptimal performance of all the rapid tests evaluated.[43] A more recent systematic review examined the accuracy of various in-house, purified antigens for serodiagnosis.[44] Although no antigen achieved sufficient sensitivity to replace sputum smear microscopy, this review helped identify several promising potential candidate antigens for an antibody detection test for pulmonary TB in patients infected and uninfected with HIV. This comprehensive review also showed that combinations of select antigens provided higher sensitivities than single antigens.[44] Several industry and academic groups are currently working on developing improved serodiagnostic tests, especially for point-of-care (POC) use.

Antigen Detection Tests

Antigen detection has the potential to overcome some of the well-recognized problems with antibody detection assays, especially in populations infected with HIV. Although several antigen detection assays have been evaluated, detection of urinary lipoarabinomannan (LAM) (a heat-stable lipoglycan in the mycobacterial cell wall) was considered a particularly good candidate, based on early studies, especially in individuals infected with HIV.[45] Early proof-of-principle data and the attractiveness of a simple urine-based TB test led to rapid commercialization of this test, initially by Chemogen Inc (Portland, ME, USA), and subsequently by Inverness Medical Innovations (Waltham, MA, USA), which marketed the test as Clearview TB enzyme-linked immunosorbent assay (ELISA). Subsequent field studies in high-burden settings have shown LAM performance to be variable and suboptimal, with lower sensitivity than expected.[46,47] However, some emerging data suggest that LAM may perform better in HIV-positive individuals with advanced immunosuppression.[48] Work is ongoing to improve and optimize the performance of LAM detection assays.

Interferon-γ Release Assays

Until recently, the diagnosis of latent tuberculosis infection depended solely on the tuberculin skin test (TST), a test with several limitations.[49] A major advance in recent times has been the development of T-cell-based interferon-γ release assays (IGRAs). IGRAs are in vitro tests that are based on interferon-γ (IFN-γ) release after T-cell stimulation by antigens (such as early secreted antigenic target 6 [ESAT6] and culture filtrate protein 10 [CFP10]) that are more specific to *M tuberculosis* than the purified protein derivative (PPD). Two IGRAs are currently available as commercial kits that are approved by the US Food and Drug Administration (FDA) and CE marked for use in Europe: the QuantiFERON-TB Gold In-Tube (QFT) assay (Cellestis Ltd., Carnegie, Australia), and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK).

Systematic reviews have reported strong evidence that IGRAs have high specificity that is unaffected by bacille Calmette-Guérin (BCG) vaccination.[50,51] TST, in contrast, has high specificity in populations who have not been vaccinated with BCG but specificity is modest and inconsistent in populations vaccinated with BCG. In low-incidence settings, IGRA results correlate well with surrogates of TB exposure. The high specificity of IGRAs is proving to be useful in individuals vaccinated with BCG, particularly in countries where TST specificity is compromised by BCG vaccination after infancy or by multiple BCG vaccinations.[49] A World Atlas of BCG Policies and Practices (**Fig. 4**) has been compiled to help clinicians and public health practitioners better interpret TST and decide on populations in which the more-specific IGRAs may be more appropriate than the TST.[52] For example, some countries recommend booster BCG shots post infancy and into adolescence, which can compromise the value of TST. IGRAs may be excellent options in these populations. The Atlas provides information on current and past policies on vaccination.

Sensitivity of IGRAs and TST is not consistent across tests and populations, but IGRAs seem to be at least as sensitive as the TST (estimated with active TB as the surrogate reference standard).[51] However, as pointed out by several investigators,[53,54] the diagnosis of active TB depends on microbiological detection of *M tuberculosis*. Immune-based tests, such as IGRAs and TST, do not directly detect *M tuberculosis*; they merely indicate a cellular immune response to recent or remote sensitization with *M tuberculosis*. Because IGRAs cannot distinguish between latent and active TB, a positive IGRA result may not necessarily indicate active TB. A negative IGRA result would not conclusively rule out active disease in an individual suspected to have TB (similar to the results of a TST).

The use of IGRAs is steadily increasing in countries with low or intermediate incidence. More than a dozen countries now have at least 1 guideline or statement on the use of IGRAs.[55] These include the United States, Canada, the United Kingdom,

Fig. 4. World Atlas of BCG Policies and Practices, http://www.bcgatlas.org. (*Courtesy of* Alice Zwerling, MSc, Montreal, Canada; with permission.)

Japan, France, Spain, Italy, Germany, Switzerland, Australia, the Netherlands, Denmark, the Czech Republic, the Slovak Republic, Korea, and Norway. In these guidelines, 3 main approaches have been recommended for the use of IGRAs: (1) TST should be replaced by IGRA; (2) either TST or IGRA may be used; (3) 2-step approach with TST first, followed by IGRA. Although the broad approach may follow 1 of these recommendations, some guidelines recommend more than 1 approach, depending on the risk group tested. For example, subgroups such as children and immunocompromised patients often receive different recommendations from other groups. **Table 2** shows the approaches recommended for use of IGRAs in several low-incidence countries.[55] As seen in the table, there is considerable diversity of how various countries currently recommend and use IGRAs. The 2-step approach seems to be the most dominant strategy and this may partly be because of cost considerations.

Despite the large number of publications on IGRAs, evidence is still limited on the prognostic value of these tests, and their added value in TB diagnosis and control.[51,56] There is growing evidence that the performance of IGRAs varies between countries with high and low incidence of TB.[57] Their role, if any, seems to be limited in low-income countries with a high TB burden, although several field evaluations are ongoing, supported by FIND and other agencies.[57]

Improved Skin Tests

A well-recognized limitation of the conventional TST is the lack of specificity of the PPD, a crude mixture with a large number of potentially cross-reacting antigens. Investigators working on this problem have attempted to replace PPD with antigens (such as ESAT6) that are specific to *M tuberculosis*. Small-scale, phase 1 trials of this improved skin test have shown promise, but further validation is needed.[58,59] Despite the limited evidence on these reagents, 1 company (Masterpharm, Russia) is already marketing a commercial product called Diaskintest (based on ESAT6/CFP10).[60] It remains to be seen if this improved skin test reagent can safely replace the conventional PPD.

POC TECHNOLOGIES

The ideal TB diagnostic test is a simple, low-technology, POC test that can be rapidly performed and yield accurate results. In 2009, a group including representatives from Médecins Sans Frontières, Treatment Action Group, Partners in Health, and other agencies, developed minimum technical test specifications that must drive the development of any new POC TB test (**Table 3**).[61] No existing test meets all of these specifications, although the Xpert MTB/RIF assay meets most of them. However, because of growing interest in new tools and biomarkers, and the increased availability of funding and grants, several agencies and groups are working on developing POC tests for TB, including improved serologic assays, detection of volatile organic compounds in breath, hand-held molecular devices, microchip technologies, and tests based on platforms such as proteomics and metabolomics.

Recently, the X PRIZE Foundation received a planning grant from the Bill & Melinda Gates Foundation to develop an X PRIZE for effective diagnosis of TB in the developing world.[62] It remains to be seen if such prize-based competitions foster innovations that deliver the POC test that will revolutionize TB diagnosis. A significant limitation on the effect of a POC test for TB is that TB is a notifiable disease that requires patient

Table 2
Recommendations from various countries that have guidelines on the use of IGRAs[a]

General Testing Approach	Countries
TST should be replaced by IGRA (ie, only IGRA is used)	Germany (anti-TNF-α therapy), Switzerland (anti-TNF-α therapy), Denmark (anti-TNF-α therapy, BCG-vaccinated contacts/adults)
Either TST or IGRA may be used	United States, France, Australia (refugees), Japan (QFT preferred in all groups except in children <5 years), Denmark (child contacts)
Two-step approach: TST first, followed by IGRA (either to improve specificity or sensitivity)	Canada, United Kingdom, Italy, Spain, Australia, the Slovak Republic, Germany (contacts), Switzerland (contacts), the Netherlands (contacts, immigrants), Norway, Korea (contacts)

[a] Some guidelines recommend more than 1 approach, depending on the risk group tested (eg, contacts, immunocompromised patients, children). The subgroups are indicated in parentheses.

Table 3
Proposed minimum set of specifications for the design of any new POC diagnostic test for TB

Test Specification	Minimum Required Value
Medical decision	Treatment initiation
Sensitivity: adults (for pulmonary TB only; regardless of HIV status)	Pulmonary TB: - 95% for smear-positive, culture-positive - (60%–)80%[a] for smear-negative, culture-positive (detection of extrapulmonary TB being a preferred but not a minimal requirement)
Sensitivity: children (including extrapulmonary TB; regardless of HIV status)	- 80% compared with culture of any specimen and - 60% of probable TB (noting problem of lack of a gold standard)
Specificity: adults	95% compared with culture
Specificity: children	- 95% compared with culture - 90% for culture-negative probable TB (noting problem of lack of a gold standard)
Time to results	3 hours maximum (patient must receive results the same day) (desirable would be less than 15 minutes)
Throughput	20 tests/d, minimum, by 1 laboratory staff member
Specimen type	Adults: urine, oral, breath, venous blood, sputum (desired: nonsputum-based sample type and use of finger prick instead of venous blood) Children: urine, oral, capillary blood (finger/heel prick)
Sample preparation	- 3 steps maximum - Safe: biosafety level 1 - Ability to use approximate volumes (ie, no need for precise pipetting) - Preparation that is not highly time sensitive
Number of samples	One sample per test
Readout	- Easy to read, unambiguous, simple "yes," "no," or "invalid" answer - Readable for at least 1 h
Waste disposal	- Simple burning or sharps disposal; no glass component - Environmentally acceptable disposal
Controls	- Positive control included in test kit - Quality control simpler and easier than with SSM
Reagents	- All reagents in self-contained kit - Kit contains sample collection device and water (if needed)
Storage/stability	- Shelf life of 24 mo, including reagents - Stable at 30°C, and at higher temperatures for shorter time periods (to be defined) - Stable in high humidity environments
Instrumentation	- If instrument needed, no maintenance required - Instrument works in tropical conditions - Acceptable replacement cost - Fits in backpack - Shock resistant
Power requirement	Can work on battery
Training	- 1 d maximum training time - Can be performed by any health worker
Cost	Less than US$10 per test after scale-up

[a] Consensus could not be reached on a definite minimum value. The group could not reach consensus for 3 test specifications: sensitivity in smear-negative adults; 60% versus 80%; diagnosis of extrapulmonary TB in adults as a minimal requirement; rejection of use of sputum as a sample. For extrapulmonary TB diagnosis in adults, the interim decision was to define this specification as highly desirable but not a minimal requirement. Similarly, for exclusion of sputum as an acceptable sample, the interim decision was to define this as highly desirable but not a minimal requirement. The group concluded that further consultation with a broader group of end users and practitioners is required to obtain further confirmation of these specifications.

From Médecins Sans Frontières. Paris Meeting on TB Point-of-Care Test Specifications. URL: http://www.msfaccess.org/TB_POC_Parismeeting/. Paris: Médecins Sans Frontières. 2009; with permission.

education, 6 months of treatment, follow-up, and contact tracing. Thus, POC testing for TB through community health workers would still result only in referral of patients to a health center, and not to direct initiation of treatment.

DIAGNOSTICS FOR CHILDHOOD TB

Childhood TB is a diagnostic challenge and although many new TB diagnostics are in progress, few have been evaluated extensively in children.[63,64] For example, several IGRA studies have been performed in adults, but few large studies exist in children. Despite the lack of strong evidence, many guidelines on IGRAs have suggested that they could be used as an adjunct tool for diagnosing TB in children,[65,66] because evidence of TB infection in children is often used in making a diagnosis of active TB, in addition to symptoms, radiological abnormalities, history of exposure, and microbiological investigations.[67] Although IGRAs may be used as a supplementary diagnostic aid in combination with the TST and other investigations to help support a diagnosis of TB, IGRAs should not be a substitute for, or obviate, appropriate specimen collection for microbiologic diagnosis.[65] Apart from IGRAs, there is a need to validate all new tools under development among children, especially young children and children infected with HIV. The poor performance of reference standard methods makes this a challenging population in which to validate the performance of a new assay.

COST-EFFECTIVENESS AND POTENTIAL IMPACT OF NEW TOOLS

For resource-poor countries with a high TB burden, the cost of introducing new tools, their successful implementation, and long-term sustainability are important concerns. Most existing studies on TB diagnostics focus on the test performance and accuracy. Few studies examine cost-effectiveness, and few model the potential impact of introduction of new diagnostic tests.

Recently, Sohn and colleagues[68] described an approach for computing the costs of TB diagnostic tests, and provided templates for various data elements and parameters that contribute to the costing analysis (**Fig. 5**). The development of a standardized methodology for costing of TB diagnostic tests would enable improved and more generalizable costing analyses, which would then provide a strong foundation for more advanced analyses that evaluate the full economic and epidemiologic impact of the implementation of validated new diagnostics.[68] This in turn should enable evidence-based adoption of new diagnostics, especially in settings with limited resources.

Dowdy and colleagues[69] used a decision-analysis model that suggested that novel diagnostic tests have the potential to be cost-effective tools in TB control. They argued that "to produce a cost-effective tool for public health, the quest for new TB diagnostics should focus on high specificity, affordability and sensitivity for cases missed by existing diagnostic standards."[69]

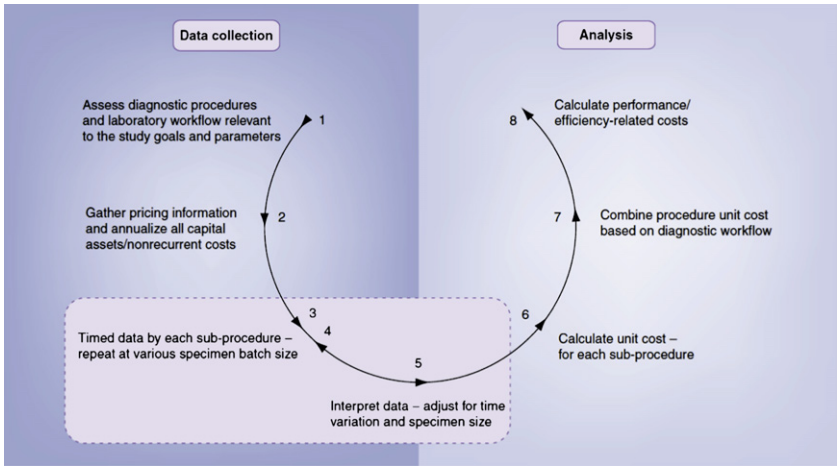


Fig. 5. Major steps involved in cost analyses of TB laboratory diagnostics. This diagram provides a step-by-step plan for cost analysis in evaluating TB diagnostic tests in various study settings. Steps 3, 4, and 5 should be undertaken for all the methods evaluated and relevant subprocedures and repeated to capture data variations caused by specimen loads (or specimen batch size). In step 7, the investigator should consult laboratory experts regarding diagnostic workflow to reflect local laboratory practice in combining procedure unit costs. (*From* Sohn H, Minion J, Albert H, et al. TB diagnostic tests: how do we figure out their costs? Expert Rev Anti Infect Ther 2009;7(6): 723–33; with permission.)

Table 4
Summary of recent modeling and cost-effectiveness studies onTB diagnostics

Study (Reference)	Diagnostic Systems Evaluated	Key Finding(s)
Keeler et al 2006[70]	Not specific: a modeling study that evaluated the effect (accounting for speed, test performance, and access) of an arbitrary new diagnostic test introduced based on current diagnostic capacity using conventional tests (sputum microscopy and chest radiograph)	• More than 400,000 lives can be saved if new innovative TB diagnostic tests can achieve 85% sensitivity for smear-positive and -negative cases, and greater than 97% specificity • New diagnostic tools should be simple, easy to use, and rapid, with results available within 1 h • There is no 1 specific solution (diagnostic tool) in achieving the best health outcome: the need for implementation of "multiple solutions"
Dowdy et al 2008[71]	Expansion of culture and DST capacity throughout South Africa	• Culture and DST on 37% of new cases and 85% of previously treated cases can save approximately 48,000 lives • This is explained as 17.2% reduction in TB mortality, 14.1% reduction in MDR-TB cases (~8000 MDR-TB cases averted) and prevention of close to 47% of MDR-TB related deaths • Expansion of culture and DST capacity can bring significant reduction in overall TB incidences and mortality in South Africa, but additional testing capacity other than culture (molecular and second line drug testing) is needed to address concerns for XDR-TB
Dowdy et al 2006[72]	Five different interventions (2 of which are nonspecific diagnostic tests: rapid molecular testing and mycobacterial culture) and their effect on TB incidence, prevalence, and steady-state population with TB-HIV coinfection problems	• Enhanced diagnostic techniques are projected to reduce TB prevalence and mortality by 20% or more, which is greater than interventions such as case-finding or antiretroviral therapy in HIV-positive patients alone • Improving TB diagnostic techniques can have a substantial effect on TB epidemiology, which in turn will provide improvement in socioeconomic factors

Dowdy et al 2008[73]	Comparison of cost-effectiveness of use of 3 different diagnostic strategies in diagnosing TB in HIV patients: (1) smear microscopy only, (2) TB culture with solid media, and (3) TB culture with liquid media	• With smear microscopy as a baseline, solid media culture can potentially avert an estimated 8 TB deaths and 37 DALY at a cost of US$962 per DALY, whereas automated liquid culture can avert 1 additional death (9 deaths) and 8 DALYs at $2751 per DALY • Higher cost per DALY associated with liquid culture is not necessarily caused by actual cost of the diagnostic test (see later discussion) • Cost-effectiveness of introduction of TB culture was more sensitive to the characteristics of the existing TB diagnostic infrastructure (communication of test results and use of these test results for clinical treatment is more important) than the test accuracy or cost
Mueller et al 2008[74]	Comparison of cost and cost-effectiveness of various liquid (automated and manual) and solid (commercially prepared and home-made) culture methodologies	• All methodologies indicate comparable costs per culture (between US$28 and $32) • Cost per *M tuberculosis* specimen detected were between US$197 and $340, where home-made solid culture method was most expensive • Liquid media brought substantially higher yields with comparable cost-effectiveness to solid culture
Acuna-Villaorduna et al 2008[75]	Comparison of cost and cost-effectiveness of 4 different rapid DST (FASTPlaque-response, direct amplification and reverse hybridization of the *rpoB* gene [INNO-LiPA], indirect colorimetric minimum inhibitory concentration assay [MTT], and direct proportion method on solid media) methods to conventional culture DST (indirect proportion method on solid media) in the context of a clinical trial	• At 2% MDR-TB prevalence direct proportion method on solid culture and MTT assays were most cost-effective with US$41 and $95 per DALY gained • Other methodologies such as FASTPlaque and INNO-LiPA (US$150 and $163 per DALY) are less cost-effective because of their higher test "kit" costs • Compared with the baseline indirect proportion method on solid culture ($156 per DALY), all rapid methodologies evaluated show comparable cost-effectiveness • Selection of rapid diagnostic technology/techniques should also take consideration of implementation issues (infrastructure capacity to introduce new technology and complexity of methodology)

Abbreviations: DALY, disability-adjusted life years; DST, drug susceptibility testing.

What impact can new diagnostics have on TB control? A few modeling studies have explored this issue (**Table 4**). Keeler and colleagues[70] reported that a rapid and widely available diagnostic for TB with sensitivity greater than 85% for smear-positive and smear-negative cases, and 97% specificity, could save approximately 400,000 lives each year. In another modeling study, Dowdy and colleagues[71] showed that expanding TB culture capacity and drug susceptibility testing in South Africa could substantially reduce TB, and particularly MDR-TB, mortality. These investigators also showed that TB cultures and new diagnostics are potentially effective and cost-effective for HIV-positive patients in diverse resource-constrained settings.[72,73] Other modeling studies have explored the use of solid versus liquid cultures,[74] and cost-effectiveness of rapid drug susceptibility testing methods.[75]

Although modeling studies are heavily dependent on the underlying assumptions and cost estimates used, they are useful for predicting the likely costs versus impact and benefits of new tools. Uncertainty in model parameters can often be addressed using sensitivity analyses. Modeling studies should be followed up with real-world studies in which new tools are introduced, to see if they really make a difference to the lives of TB patients; this is often done in field demonstration studies, as part of routine NTP services. Implementation and operations research are therefore highly desirable whenever new tools and interventions are introduced in programmatic conditions. Indeed, they are essential for evidence-based selection and implementation of diagnostic tools in the global strategy to control TB.

REFERENCES

1. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis 2007;196(Suppl 1):S15–27.
2. Pai M, O'Brien R. New diagnostics for latent and active tuberculosis: state of the art and future prospects. Semin Respir Crit Care Med 2008;29:560–8.
3. Sreeramareddy CT, Kishore PV, Menten J, et al. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. BMC Infect Dis 2009;9:91.
4. Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics for developing countries. Lancet 2006;367:942–3.
5. Perkins MD, Small PM. Partnering for better microbial diagnostics. Nat Biotechnol 2006;24:919–21.
6. Minion J, Zwerling A, Pai M. Diagnostics for tuberculosis: what new knowledge did we gain through the International Journal of Tuberculosis and Lung Disease in 2008? Int J Tuberc Lung Dis 2009;13:691–7.
7. Stop TB Partnership, World Health Organization. The global plan to stop TB 2006–2015. Geneva (Switzerland): World Health Organization; 2006.
8. Pai M, Ramsay A, O'Brien R. Evidence-based tuberculosis diagnosis. PLoS Med 2008;5:e156.
9. World Health Organization. Moving research findings into new WHO policies. Geneva (Switzerland): World Health Organization; 2008. Available at: http://www.who.int/tb/dots/laboratory/policy/en/index4.html. Accessed October 1, 2009.
10. World Health Organization, Stop TB Partnership. New laboratory diagnostic tools for tuberculosis control. In: Geneva (Switzerland): World Health Organization; 2008.
11. Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006;6:570–81.
12. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006;6:664–74.
13. 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.
14. Mase SR, Ramsay A, Ng V, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. Int J Tuberc Lung Dis 2007;11:485–95.
15. Cambanis A, Yassin MA, Ramsay A, et al. A one-day method for the diagnosis of pulmonary tuberculosis in rural Ethiopia. Int J Tuberc Lung Dis 2006;10:230–2.
16. Minion J, Sohn H, Pai M. Light-emitting diode technologies for TB diagnosis: what's on the market? Expert Rev Med Devices 2009;6(4):341–5.
17. Breslauer DN, Maamari RN, Switz NA, et al. Mobile phone based clinical microscopy for global health applications. PLoS One 2009;4:e6320.
18. Sadaphal P, Rao J, Comstock GW, et al. Image processing techniques for identifying *Mycobacterium tuberculosis* in Ziehl-Neelsen stains. Int J Tuberc Lung Dis 2008;12:579–82.
19. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007;11:1–178.
20. Cruciani M, Scarparo C, Malena M, et al. 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.
21. Ngamlert K, Sinthuwattanawibool C, McCarthy KD, et al. Diagnostic performance and costs of Capilia TB for *Mycobacterium tuberculosis* complex identification from broth-based culture in Bangkok, Thailand. Trop Med Int Health 2009;14:748–53.

22. World Health Organization. The use of liquid medium for culture and DST. Geneva (Switzerland): World Health Organization; 2007. Available at: http://www.who.int/tb/dots/laboratory/policy/en/index3.html. Accessed October 1, 2009.
23. Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N Engl J Med 2006;355:1539–50.
24. Martin A, Munga Waweru P, Babu Okatch F, et al. Implementation of the thin layer agar for the diagnosis of smear-negative pulmonary tuberculosis in a high HIV prevalence setting in Homa Bay, Kenya. J Clin Microbiol 2009;47:2632–4.
25. Shikama ML, Ferro e Silva R, Villela G, et al. Multicentre study of nitrate reductase assay for rapid detection of rifampicin-resistant *M. tuberculosis*. Int J Tuberc Lung Dis 2009;13:377–80.
26. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. Eur Respir J 2005;26:339–50.
27. Bwanga F, Hoffner S, Haile M, et al. Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. BMC Infect Dis 2009;9:67.
28. Palomino JC. Molecular detection, identification and drug resistance detection in *Mycobacterium tuberculosis*. FEMS Immunol Med Microbiol 2009;56:103–11.
29. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep 2009;58:7–10.
30. Greco S, Girardi E, Navarra S, et al. The current evidence on diagnostic accuracy of commercial based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax 2006;61:783–90.
31. Ling DI, Flores LL, Riley LW, et al. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One 2008;3:e1536.
32. Pai M, Flores LL, Hubbard A, et al. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. BMC Infect Dis 2004;4:6.
33. Pai M, Flores LL, Pai N, et al. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect Dis 2003;3:633–43.
34. 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. J Clin Microbiol 2007;45:1936–40.
35. Cepheid announces new diagnostic technology in ongoing efforts to halt the spread of TB. Available at: http://www.finddiagnostics.org/export/sites/default/media/press/090324.html. Foundation for Innovative New Diagnostics, 2009. Accessed June 17, 2009.
36. Morgan M, Kalantri S, Flores L, et al. A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. BMC Infect Dis 2005;5:62.
37. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J 2008;32:1165–74.
38. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol 2009;47:1767–72.
39. Policy statement. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Available at: http://www.who.int/tb/features_archive/policy_statement.pdf. World Health Organization. Accessed October 1, 2009.
40. UNITAID approves over US $61 million to GLI, FIND and GDF for MDR-TB diagnostics. Available at: http://www.finddiagnostics.org/export/sites/default/media/news/090518.html. Foundation for Innovative New Diagnostics, 2009. Accessed June 17, 2009.
41. Steingart KR, Henry M, Laal S, et al. A systematic review of commercial serological antibody detection tests for the diagnosis of extra-pulmonary tuberculosis. Thorax 2007;62:911–8.
42. Steingart KR, Henry M, Laal S, et al. Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: a systematic review. PLoS Med 2007;4:e202.
43. World Health Organization. Diagnostics Evaluation Series No.2. Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis. Geneva (Switzerland): World Health Organization; 2008.
44. Steingart KR, Dendukuri N, Henry M, et al. Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: a meta-analysis. Clin Vaccine Immunol 2009;16:260–76.
45. Boehme C, Molokova E, Minja F, et al. Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis. Trans R Soc Trop Med Hyg 2005;99:893–900.
46. Daley P, Michael JS, Hmar P, et al. Blinded evaluation of commercial urinary lipoarabinomannan for active tuberculosis: a pilot study. Int J Tuberc Lung Dis 2009;13(8):989–95.
47. Mutetwa R, Boehme C, Dimairo M, et al. Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African TB suspects and patients. Int J Tuberc Lung Dis 2009;13(10):1253–9.

48. Lawn SD, Edwards D, Kranzer K, et al. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. AIDS 2009;23(14):1875–80.
49. Farhat M, Greenaway C, Pai M, et al. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis 2006;10:1192–204.
50. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007;146:340–54.
51. Pai M, Zwerling A, Menzies D. T-cell based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008;149:177–84.
52. Zwerling A, Behr M, Brewer T, et al. Which countries are most likely to benefit from highly specific IGRAs? Findings from the World Atlas of BCG Policies and Practices. Am J Respir Crit Care Med 2009;179: A4773.
53. Lange C, Pai M, Drobniewski F, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: sensible or silly? Eur Respir J 2009; 33:1250–3.
54. Pai M, Menzies D. Interferon-gamma release assays: what is their role in the diagnosis of active tuberculosis? Clin Infect Dis 2007;44:74–7.
55. Pai M. Guidelines on IGRAs: concordant or discordant? In: 2nd Global symposium on IGRAs; May 30–June 1, 2009; Dubrovnik, Croatia: 2009.
56. Pai M, Dheda K, Cunningham J, et al. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis 2007;7:428–38.
57. Dheda K, Smit RZ, Badri M, et al. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. Curr Opin Pulm Med 2009;15:188–200.
58. Arend SM, Franken WP, Aggerbeck H, et al. Double-blind randomized Phase I study comparing rdESAT-6 to tuberculin as skin test reagent in the diagnosis of tuberculosis infection. Tuberculosis 2008;88:249–61.
59. Wu X, Zhang L, Zhang J, et al. Recombinant early secreted antigen target 6 protein as a skin test antigen for the specific detection of *Mycobacterium tuberculosis* infection. Clin Exp Immunol 2008;152:81–7.
60. Kiselev VI, Baranovsky PM, Rudykh IV, et al. [Clinical trials of the new skin test Diaskintest for the diagnosis of tuberculosis]. Probl Tuberk Bolezn Legk 2009;2:11–6 [in Russian].
61. Paris Meeting on TB Point-of-Care Test Specifications. Available at: http://www.msfaccess.org/TB_POC_Parismeeting/. Médecins Sans Frontières, 2009. Accessed June 17, 2009.
62. X PRIZE Foundation to Help Fight Tuberculosis Worldwide with Gates Foundation Support. URL.http://www.xprize.org/foundation/press-release/x-prize-foundation-to-help-fight-tuberculosis-worldwide-with-gates-foundati. XPrize Foundation, 2008. Accessed June 17, 2009.
63. Marais BJ, Gie RP, Schaaf HS, et al. Childhood pulmonary tuberculosis: old wisdom and new challenges. Am J Respir Crit Care Med 2006;173: 1078–90.
64. Marais BJ, Pai M. New approaches and emerging technologies in the diagnosis of childhood tuberculosis. Paediatr Respir Rev 2007;8:124–33.
65. Updated recommendations on interferon gamma release assays for latent tuberculosis infection. An Advisory Committee Statement (ACS). Can Commun Dis Rep 2008;34:1–13.
66. Mazurek GH, Jereb J, Lobue P, et al. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. MMWR Recomm Rep 2005;54:49–55.
67. Hopewell PC, Pai M, Maher D, et al. International standards for tuberculosis care. Lancet Infect Dis 2006;6:710–25.
68. Sohn H, Minion J, Albert H, et al. TB diagnostic tests: how do we figure out their costs? Expert Rev Anti Infect Ther 2009;7(6):723–33.
69. Dowdy DW, O'Brien MA, Bishai D. Cost-effectiveness of novel diagnostic tools for the diagnosis of tuberculosis. Int J Tuberc Lung Dis 2008;12: 1021–9.
70. Keeler E, Perkins MD, Small P, et al. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature 2006;444(Suppl 1):49–57.
71. Dowdy DW, Chaisson RE, Maartens G, et al. Impact of enhanced tuberculosis diagnosis in South Africa: a mathematical model of expanded culture and drug susceptibility testing. Proc Natl Acad Sci U S A 2008; 105:11293–8.
72. Dowdy DW, Chaisson RE, Moulton LH, et al. The potential impact of enhanced diagnostic techniques for tuberculosis driven by HIV: a mathematical model. AIDS 2006;20:751–62.
73. Dowdy DW, Lourenco MC, Cavalcante SC, et al. Impact and cost-effectiveness of culture for diagnosis of tuberculosis in HIV-infected Brazilian adults. PLoS One 2008;3:e4057.
74. Mueller DH, Mwenge L, Muyoyeta M, et al. Costs and cost-effectiveness of tuberculosis cultures using solid and liquid media in a developing country. Int J Tuberc Lung Dis 2008;12:1196–202.
75. Acuna-Villaorduna C, Vassall A, Henostroza G, et al. Cost-effectiveness analysis of introduction of rapid, alternative methods to identify multidrug-resistant tuberculosis in middle-income countries. Clin Infect Dis 2008;47:487–95.