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Tuberculosis Diagnostics in 2015: Landscape, Priorities, Needs, and Prospects

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TUBERCULOSIS DIAGNOSTICS IN 2015: LANDSCAPE, PRIORITIES, NEEDS, AND PROSPECTS

SUPPLEMENT ARTICLES

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Tuberculosis Diagnostics in 2015: Landscape, Priorities, Needs, and Prospects

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In 2015, tuberculosis remains a major global health problem, and drug-resistant tuberculosis is a growing threat. Although tuberculosis diagnosis in many countries is still reliant on older tools, new diagnostics are changing the landscape. Stimulated, in part, by the success and roll out of Xpert MTB/RIF, there is now considerable interest in new technologies. The landscape looks promising, with a robust pipeline of new tools, particularly molecular diagnostics, and well over 50 companies actively engaged in product development. However, new diagnostics are yet to reach scale, and there needs to be greater convergence between diagnostics development and development of shorter-duration tuberculosis drug regimens. Another concern is the relative absence of non–sputum-based diagnostics in the pipeline for children and of biomarker tests for triage, cure, and progression of latent *Mycobacterium tuberculosis* infection. Several initiatives, described in this supplement, have been launched to further stimulate product development and policy, including assessment of needs and priorities, development of target product profiles, compilation of data on resistance-associated mutations, and assessment of market size and potential for new diagnostics. Advocacy is needed to increase funding for tuberculosis research and development, and governments in high-burden countries must invest more in tuberculosis control to meet post-2015 targets for care, control, and prevention.

Keywords. tuberculosis; diagnostics; pipeline; unmet needs; market potential.

While much progress has been made with tuberculosis control, the World Health Organization (WHO) estimates that 9 million people developed tuberculosis in 2013 and that 1.5 million died, including 360 000 people who were infected with human immunodeficiency virus (HIV; Figure 1) [1]. Rapid, accurate diagnosis is critical for timely initiation of antituberculosis treatment, but many people with tuberculosis (or tuberculosis symptoms) do not have access to adequate initial diagnosis. In 2013, >3 million cases were missed by the health system, either because they were not diagnosed or were not notified to national tuberculosis programs [1].

Access to adequate diagnosis is particularly poor for patients with multidrug-resistant (MDR) tuberculosis

and in cases of childhood tuberculosis. Globally, in 2013, the WHO estimated that 480 000 people developed MDR tuberculosis [1]. However, only 136 000 MDR tuberculosis cases were detected, with second-line treatment initiated for 97 000. Also, in 2013, an estimated 535 000 children developed tuberculosis, but the true case burden of childhood tuberculosis is likely higher. A model-based estimate suggests that the number was closer to 1 million children in 2010 [2]. Childhood tuberculosis is very difficult to diagnose, and most conventional tuberculosis tests perform poorly in this high-risk population.

In 2014, the WHO and partners announced a post-2015 tuberculosis strategy and accompanying targets with the goal of ending the global tuberculosis epidemic [3]. This ambitious strategy aims to reduce the tuberculosis incidence by 90% by 2035 (compared with the 2015 incidence). Early diagnosis of tuberculosis, including universal drug-susceptibility testing (DST), and systematic screening (active case finding) of contacts and high-risk groups are key components of this new strategy. Discovery, development, and rapid uptake of new

Figure 1. Status of the tuberculosis problem in 2014. The graphic is reproduced with permission from the World Health Organization (http://www.who.int/tb/features_archive/globaltb_report2014/en/).

tools, interventions, and strategies are also highlighted as important components [3].

LANDSCAPE AND PIPELINE OF TUBERCULOSIS DIAGNOSTIC TECHNOLOGIES

Although tuberculosis diagnosis in 2014 is still reliant on older tools such as smear microscopy and culture, new diagnostics are changing the tuberculosis diagnostics landscape. Worldwide, the ongoing roll out of Xpert MTB/RIF (Cepheid, Sunnyvale, California) continues to be the most important, measurable shift in the tuberculosis diagnostics landscape. According to the WHO, as of 30 September 2014, 3553 GeneXpert instruments (comprising >17 000 modules) and 8.8 million Xpert MTB/RIF cartridges had been procured by the public sector in 110 of 145 countries eligible for concessional pricing [4]. The Xpert technology is significantly more sensitive than sputum smear microscopy and can also rapidly detect rifampicin resistance with high accuracy [5].

Stimulated, in part, by the success and roll out of Xpert MTB/RIF, there is now considerable interest in new tuberculosis diagnostics. The 2014 UNITAID TB Diagnostics Technology and Market Landscape report summarized the technologies that have been endorsed by the WHO and described the pipeline of novel tools that are on or likely to enter the market [6]. As described in the UNITAID report and summarized by stakeholders such as the Foundation for Innovative New Diagnostics, the landscape looks promising, with a robust pipeline of new tools and well over 50 companies actively engaged in product development. Figure 2 shows the pipeline of tools and the expected complexity of the products under development.

In the short term, the most impressive trend is the expansion of the range of molecular technologies that could potentially replace smear microscopy [6]. As shown in Figure 3, new molecular products on the market (or in the pipeline) will compete with the Xpert technology, and some may be deployable in peripheral microscopy centers, where millions of patients are tested. This level of decentralized deployment is feasible but challenging with the Xpert technology because of technical and infrastructure issues [7–10].

In addition to rapid case detection, newer molecular tools will have the capacity to identify drug-resistance mutations and thereby help countries reach the post-2015 target of universal DST for all patients with tuberculosis, at the time of detection. With the impending introduction of new tuberculosis drug regimens (described below), this is of great significance. New drug regimens will require companion diagnostics to ensure rapid completion of the so-called test and treat approach. While newer molecular diagnostics are ideally suited to serve the role of companion diagnostics to new drug regimens, a major hurdle is the lack of high-quality validation studies of newer molecular tests. Several assays are now on the market with virtually no validation trials published on their accuracy and performance. This suggests the need for ensuring global and country-level systems for rapid validation of new tools, to ensure that such evidence is translated into policies.

In the medium term, the need for a biomarker-based, low-cost, non–sputum-based test remains an important priority for tuberculosis diagnostics at the primary care level, where the majority of people first seek care [6]. Although biomarker discovery is an active area and several potential products (eg, antigen or antibody detection tests, volatile organic compound analysis, and enzymatic detection) are under development, no test under development is likely to be on the market with policy endorsements within the next 3–5 years [11].

In the longer term, a breakthrough in biomarker discovery is necessary to identify those with latent *Mycobacterium tuberculosis* infection who are at the highest risk of progressing to tuberculosis, so that the vast pool of latently infected individuals can be successfully reduced [6]. Since molecular tests are usually not helpful for treatment monitoring, a biomarker-based test

Global TB diagnostic pipeline

	Early Development	Late or Completed Development	On Pathway to WHO Evaluation
HIGH COMPLEXITY ASSAYS			
Molecular Detection/DST	TruArray MDR-TB (Akkoni) COBAS TaqMan MTB +DST(Roche) Hydra 1K (insilixa) Mycobacterium Real-time MDR (CapitalBio)	TRC Rapid MTB (Tosoh) VereMTB (Veredus Laboratories) LiPA Pyrazinamide (Nipro) LATE-PCR Lights on / Lights off (Hain) TBMDx (Abbott) Meltpro (Zeesan) Mycobacteria RT PCR (CapitalBio) REBA MTB-XDR (YD Diagnostics) EasyNAT TB (Ustar) BD Max (BD)	GenoTYPE MTBDRsl (Hain) LiPA MDR-TB (Nipro) REBA MTB-Rifa (YD Diagnostics)
Culture-based Technology	BNP Middlebrook (NanoLogix) Rapid colorimetric DST	TREK Sensitive MYCOTB (Trek)	
MODERATE COMPLEXITY ASSAYS			
Molecular Detection/DST	Xpert Ultra and Xtend XDR (Cepheid) Alere Q (Alere) Enigma ML (Enigma Diagnostics) Q-POC (QuantuMDx) EOSCAPE (Wave80) RT-PCR Testing Platform (NWGHF/Guidel) iCubate 2.0 (iCubate) TBDx system (KGI) DiagCORE (STAT Diagnostica) LabChip G2-3 (Nanobiosys)	Genedrive MTB/RIF (Epistem) Truelab/Truenat MTB (Molbio)	TB LAMP (Eiken)
Volatile Organic Compounds	BreathLink (Menssana) Prototype breathanalyzer (Next Dimensions) TB Breathalyser (Rapid Biosensor Systems) Aeonose (The eNose Company) Breath analysis instrument (Metabolomx)	Giant African Pouch Rats (Apopo)	
Automated Microsvopy & Imaging	TBDx (Applied Visual Sciences) Fluorescent microscopy (ID-FISH Tech.) Automatic TB Screener (Fluorobot)	Microimager (BD) CAD4TB (Delft Imaging Systems)	
LOW COMPLEXITY ASSAYS			
Antigen & Antibody Detection	LAM in sputum (Standard Diagnostics) Multiplex antibody array (mBio)		Alere Determine TB-LAM in urine (Alere)
Enzymatic Detection	β-lactamase reporter (Global BioDiagnostics)		

Source: FIND, Geneva

Figure 2. Current tuberculosis diagnostics pipeline listing the development phases and the types of technologies in development or evaluation. Complexity categorization was based on criteria that are used for similar diagnostics by the US Food and Drug Administration. Early development refers to prototype development after the proof-of-concept stage. Late-stage development refers to turning the prototype into a design-locked, manufacturable product. The graphic is reproduced with permission from the Foundation for Innovative New Diagnostics.

for cure will also be enormously helpful. The pipeline for such tests is currently weak, with few companies working on biomarker discovery to support research and development of such products. However, governmental and nongovernment organizations continue to fund the search for new biomarkers useful to meet the diagnostic, prognostic, and treatment monitoring needs.

NEEDS AND PRIORITIES

The ongoing roll out of Xpert MTB/RIF has had a positive influence on the tuberculosis diagnostics landscape, has attracted new investments and product developers, and has created a robust pipeline of technologies [6]. It has also ploughed the way for wider access to molecular tests and universal DST and prepared the ground for the next wave of innovative technologies. Lessons learned from Xpert implementation will be invaluable for scaling up next-generation technologies [9, 10].

However, the Xpert technology was not designed to reach lower tiers of the healthcare system or to meet all needs (eg, it cannot detect latent *M. tuberculosis* infection or resistance against multiple drugs). Despite initiatives to reduce the price, high cost continues to be a hurdle for underfunded national tuberculosis programs [12]. A recent survey of 22 countries with a
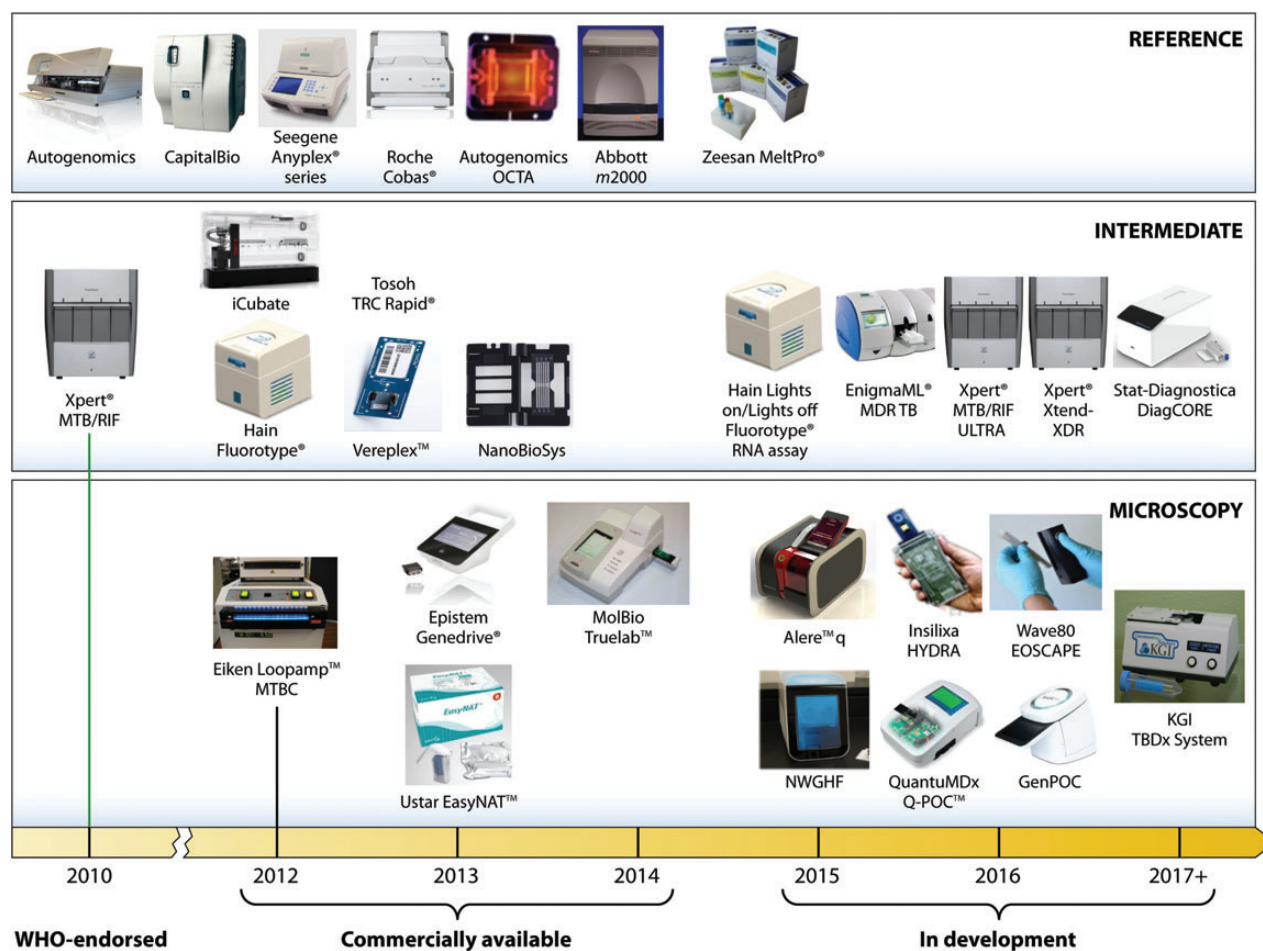
Figure 3. Pipeline of molecular diagnostics for tuberculosis, by level of deployment (ie, reference, intermediate, and peripheral microscopy laboratories). The graphic is reproduced with permission from the UNITAID (http://unitaid.org/images/marketdynamics/publications/UNITAID_TB_Diagnostics_Landscape_3rd-edition.pdf).

high tuberculosis burden (HBCs) showed that, while a majority (86%) of these countries have a policy or algorithm for use of Xpert technology, current implementation is mostly donor funded, largely dependent on testing in centralized laboratories, and primarily involves patients with presumed drug-resistance or HIV infection [13]. The survey used the ratio of smear volumes for initial diagnosis to the number of Xpert cartridges procured during a roughly similar period as an approximate index of Xpert market penetration in the public sector. The ratio in South Africa was 1.6, significantly lower than most other HBCs, where approximately 40–70 smears were performed for each Xpert cartridge [13]. This suggests that wide-scale implementation of Xpert technology has mostly occurred in South Africa, while other HBCs continue to rely heavily on smear microscopy.

A recent published study of various stakeholders helped establish the most important unmet needs and identify tools that are of highest importance. Kik et al conducted a priority-setting exercise to identify the highest priority tests for target product profile (TPP) development and investment in research and development [14]. For each of the potential TPPs, 10 criteria were used to set priorities, including prioritization by key stakeholders (eg, managers of national tuberculosis programs), potential impact of the test on tuberculosis transmission, morbidity and mortality, market potential, and implementation and scalability of the test. On the basis of this analysis, the following were identified as the highest priorities: (1) a point-of-care sputum-based test as a replacement for smear microscopy (ie, a smear-replacement test); (2) a point-of-care, non–sputum-based test capable of detecting all forms of tuberculosis via the identification of characteristic biomarkers or biosignatures (ie, a non–sputum based biomarker test); (3) a point-of-care triage test, which should be a simple, low-cost test for use by first-contact healthcare providers as a test for ruling out tuberculosis (ie, a triage test); and (4) rapid DST at microscopy centers (ie, a rapid DST).

Given the variety of unmet needs [14] and the diversity of sites where testing can occur [15], it is important for product developers to have access to (1) a clearly identified list of diagnostics that are considered high priority by the tuberculosis community; (2) well-developed, detailed TPPs for priority diagnostics, based on a consensus-building process; and (3) up-to-date market size estimations for the priority TPPs [16, 17]. These issues are addressed in subsequent articles in this supplement. The article by Denkinger et al [18, 19] describes the final TPPs that have been developed for the highest priority tests and reviewed in a consensus meeting hosted by the WHO and partners, while the articles by Kik et al [20] and Pantoja et al [21] describe the potential future market for new assays the and affordability of new tests by countries, respectively.

ALIGNMENT OF DIAGNOSTICS WITH NOVEL TUBERCULOSIS TREATMENT REGIMENS

In a recent analysis, Wells et al outlined the need for a better alignment (or convergence) between new tuberculosis diagnostics with the likely tuberculosis treatment landscape in the next 3–4 years [22]. Because of promising results in phase 2 trials, the Global Alliance for TB Drug Development and partners have launched the Shortening Treatment by Advancing Novel Drugs trial of the PaMZ drug regimen, which contains pretomanid (previously called PA-824), moxifloxacin, and pyrazinamide. If the trial is successful, by 2018, this could reduce the duration of tuberculosis therapy to 4 months [23].

For the PaMZ regimen to be implemented successfully, it is important to ensure that existing molecular diagnostics are more widely used and to develop next-generation molecular assays that can detect resistance to markers that are aligned with novel regimens such as PaMZ. This means that product developers will need better data about the molecular mechanisms of resistance. Efforts are underway (described elsewhere in this supplement by Solomon et al [24]) to develop a database of mutations associated with drug resistance and to develop strain collections to enable assessment of new diagnostic assays.

There are other new drugs, such as bedaquiline and delamanid, that have already received partial regulatory approval for use in treating MDR tuberculosis [25]. Linezolid, although not approved for MDR tuberculosis, is already being used in the field [26]. Phenotypic resistance tests for these drugs have not been established, and careful monitoring needs to take place before critical concentrations are selected on the basis of clinical data. Even though these may be new drugs to treat tuberculosis, the mechanisms of action are either similar to those of existing drugs (as is the case between bedaquiline and clofazimine), background resistance already exists (as in the case of linezolid), or they are in the same class of drugs (eg, nitroimidazoles). Thus, it will be important to monitor for drug resistance during treatment. This will be especially important for treatment of extensively drug-resistant (XDR) tuberculosis, since the number of effective drugs available is much smaller. With such limited choices, the likelihood of treating patients with XDR or pre-XDR tuberculosis with a suboptimal regimen becomes much higher. As a result, this also increases the chance of developing resistance to the remaining active drug(s), thus reducing the effectiveness of new compounds in our toolbox.

Also, as part of prelaunch activities, it is important for countries to establish sample collection and transport systems, laboratory information management systems, mechanisms for external quality assurance for molecular and DST tools, and information and communication technologies for rapid reporting of results, case notification and linkages to care, and supply chain and logistics management [27]. Greater use of existing tests (like Xpert technology, liquid cultures, and line probe assays) and drug regimens will enable national tuberculosis programs to develop and fine-tune these systems and then transition to newer drug regimens and companion diagnostics by 2018.

ONGOING EFFORTS TO IMPROVE CHILDHOOD TUBERCULOSIS DIAGNOSIS

Although identifying tuberculosis cases continues to be a challenge in adults, active tuberculosis in several special populations, including pediatric patients, is more difficult to diagnose because of extrapulmonary involvement, paucibacillary aspects, or nonspecific presentation. In low-income and middle-income countries, difficulties arise towing to the similarity of symptoms to other common diseases, including bacterial pneumonia and viral infections, and to comorbid conditions, such as malnutrition. As a result, tuberculosis treatment is often performed empirically, which leads to underdiagnosis or, in some cases, to overdiagnosis and subsequent inappropriate prescription of drugs to patients without infection. Underdiagnosis leads to increased morbidity and mortality due to tuberculosis. Overdiagnosis results higher treatment costs to tuberculosis programs and potentially contributes to the development of drug resistance due to poor adherence. This is further complicated by the fact that the time to symptom resolution in young children treated for tuberculosis requires >2 months in the majority of cases [28]. As a result, symptom-based diagnosis may not resolve when these patients are receiving tuberculosis treatment and may suggest MDR tuberculosis. Additional clinical evaluations would be needed to determine the etiology or whether to consider switching to a drug-resistant tuberculosis regimen.

Despite the need for better diagnostics, funding for pediatric diagnostics is woefully inadequate compared with that for adult diagnostics, which itself continues to lag behind funding for HIV diagnostics. Unfortunately, diagnosis and treatment is not a priority for many funding organizations since pediatric

tuberculosis has a limited impact on disease at the population level. Therefore, control of tuberculosis in children is considered to be of limited programmatic value. The original directly observed treatment, short-course strategy was heavily focused on identifying infectious cases by use of sputum smears, and this led to national tuberculosis programs placing greater emphasis on adults.

Despite these challenges, interest in diagnosing and treating tuberculosis in children has gained momentum over the past few years. This includes standardizing case definitions of tuberculosis in children [29], developing and manufacturing first-line tuberculosis drugs in appropriate child-friendly formulations (through the Global Drug Facility), and inclusion of children in clinical trials [30]. This last point is significant because disease end points, pathogenesis, and drug metabolism is different in children and infants, compared with adults [30]. Several funding institutions have recently supported research initiatives to identify new biomarkers that could be used to diagnose tuberculosis in children. These biomarkers include a combination of biological measurements at the protein or genomic level that reflect an interaction between the host and the pathogen [31, 32].

As the results of these investments become available, a greater need will be placed on further evaluating potential biomarkers, using a set of well-characterized and highly pedigreed samples. Unfortunately, standard sets of samples from children exposed to and suspected of having tuberculosis are not widely available. Although many private collections exist, standardized definitions, collection, processing, and storage of samples have not been adopted. Consequently, evaluations of potential diagnostic biomarkers may be discrepant despite the use of existing pediatric samples. Moreover, additional challenges in documenting tuberculosis exposures with clinical symptoms consistent with infection and lack of funding have hampered current efforts to store these samples in biorepositories. In addition, low sample volumes typically obtained from children and infants prevent wide dissemination of material to large numbers of investigators. Finally, there is a need not only for well-defined samples from children with tuberculosis, but of samples from children in tuberculosis-endemic areas who have clinical signs consistent with tuberculosis but are free of the disease. This is most critical because the performance of a biomarker will need to be able to differentiate *M. tuberculosis* infection from a number of other conditions that typically present with similar clinical signs in tuberculosis-endemic areas.

MARKET FOR NEW TECHNOLOGIES

Product developers need information on market size and potential, to make investment decisions [17]. A recent series of studies have tried to quantify the current served available market value of tuberculosis diagnostics. A survey of 22 HBCs showed that they performed 77.6 million sputum smears in >42 000 microscopy centers annually, with a cost of $137 million [33]. Of these, 61% were performed in the BRICS countries. A detailed analysis of what Brazil spent on tuberculosis diagnosis showed that, during 2012, an estimated 2.4 million tuberculosis diagnostic tests were conducted, resulting in an estimated overall market value of $17.2 million [34]. The public sector accounted for 91% of the test volume and 88% of the market value. Smear microscopy was the most commonly used test (1.3 million tests [55%]), with an estimated cost of $3.7 million. A total of 302 761 cultures were performed, representing 13% of the test volume and 40% ($6.9 million) of the market value. On average, $208 was spent on tuberculosis diagnostics for every Brazilian patient with notified tuberculosis during 2012 [34].

Another analysis estimated the expenditure on tuberculosis diagnosis in South Africa during 2012–2013 [35]. This study showed that South Africa has a sizeable tuberculosis diagnostic market in terms of volume and value. In 2012, during Xpert scale-up, the public and private sectors performed 9.2 million tuberculosis diagnostic tests, with an estimated total cost of $98 million. The public sector accounted for 93% of the overall test volume and value, with microscopy and culture accounting for the majority of tests performed. In 2013, the public sector market value increased to $101 million (a 10% increase over 2012). While Xpert volumes increased by 166%, total tuberculosis test volumes decreased by 12%, compared with 2012 values [35]. Similar analyses are being completed for China and India.

On the basis of these analyses, Kik et al [20] made projections about the potential available market for the 4 priority TPPs that have been developed. They found that, of the 4 TPPs, the greatest potential available market in terms of value would be for a sputum-based tuberculosis detection and DST upfront test. A test that can be deployed at lower levels of the healthcare system and used for detecting (or ruling out) all forms of tuberculosis, such as a biomarker test or a triage test, would have the largest potential market volume.

The publication of technology and market landscape reports, TPPs, and market size estimates are all intended to stimulate increased investments in the area of tuberculosis diagnostics. While the overall trend is positive (as seen in the number of products and companies), tuberculosis research and development as a whole continues to be severely underfunded.

FUNDING FOR TUBERCULOSIS RESEARCH AND DEVELOPMENT AND FOR PRODUCT EVALUATION

A 2014 annual research and development funding report by Treatment Action Group, showed that the world invested only one third of the required $2 billion needed every year for new

drugs, diagnostics, and vaccines to fight the global tuberculosis epidemic effectively [11]. In 2013, $676.6 million was spent on tuberculosis research. Of the $9.8 billion in funding required for tuberculosis research during 2011–2015, as estimated by The Global Plan to Stop TB, only 20% of this amount has been mustered at the end of 2013. The Treatment Action Group report registered a significant funding shortfall across every category of tuberculosis research: basic science, diagnostics, drugs, vaccines, and operational research. The report also showed that, during 2013, research and development spending by pharmaceutical companies for tuberculosis was among the lowest recorded levels. These funding trends have great consequences for biomarker and basic research work that is critically important for novel tuberculosis tests and biomarkers for childhood and extrapulmonary tuberculosis, markers for treatment monitoring, and markers for predicting progression from latent *M. tuberculosis* infection to tuberculosis. In addition to increasing funding for research and development, donors, governments, and private industry must find a way to increase funding for product evaluation. Otherwise, we may see a plethora of new tools with few data to support or refute their incorporation into policy.

CONCLUSIONS

In 2015, the tuberculosis diagnostics landscape looks promising, with a robust pipeline and several companies actively engaged. However, new diagnostics have yet to reach scale, and there needs to be greater alignment between diagnostics and novel tuberculosis drug regimens. While the pipeline is robust for molecular tools, the pipeline is less robust for other products, especially biomarker-based tests for cure, triage, and predicting progression of latent *M. tuberculosis* infection. Several initiatives, described in this supplement, are ongoing to stimulate product development and policy, including assessment of needs and priorities, development of TPPs, compilation of data on resistance-associated mutations, and assessment of market potential for new diagnostics. If these initiatives are complemented with increased advocacy for funding for tuberculosis research and development with greater engagement of countries in evaluation of new tools, and if governments in HBCs actively scale-up new diagnostics and drug regimens, it will help make the post-2015 vision of a tuberculosis-free world a reality.

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Defining the Needs for Next Generation Assays for Tuberculosis

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To accelerate the fight against tuberculosis, major diagnostic challenges need to be addressed urgently. Post-2015 targets are unlikely to be met without the use of novel diagnostics that are more accurate and can be used closer to where patients first seek care in affordable diagnostic algorithms.

This article describes the efforts by the stakeholder community that led to the identification of the high-priority diagnostic needs in tuberculosis. Subsequently target product profiles for the high-priority diagnostic needs were developed and reviewed in a World Health Organization (WHO)-led consensus meeting.

The high-priority diagnostic needs included (1) a sputum-based replacement test for smear-microscopy; (2) a non-sputum-based biomarker test for all forms of tuberculosis, ideally suitable for use at levels below microscopy centers; (3) a simple, low cost triage test for use by first-contact care providers as a rule-out test, ideally suitable for use by community health workers; and (4) a rapid drug susceptibility test for use at the microscopy center level.

The developed target product profiles, along with complimentary work presented in this supplement, will help to facilitate the interaction between the tuberculosis community and the diagnostics industry with the goal to lead the way toward the post-2015 global tuberculosis targets.

Keywords. tuberculosis; diagnosis; target product profiles; prioritization; point-of-care.

In 2012, there were an estimated 9 million tuberculosis cases leading to 1.5 million deaths, the majority of which were preventable with existing treatments if diagnosed early [1]. Major gains have been made in the fight against tuberculosis over the past decades, and the world is on track to meet the targets of the 2015 UN Millennium Development Goal of reversing tuberculosis incidence. Also, all regions except for Africa and Europe are on track to achieve a reduction in the mortality rate by 50%. However, to accelerate the fight against tuberculosis and move towards post-2015 targets and finally elimination of this disease, two major challenges need to be addressed urgently: (1) Each year 3 million patients, about one third of all tuberculosis cases, are not diagnosed or notified; (2) The emergence of drug resistance against the main anti-tuberculous drugs is creating a public health crisis in many countries around the world.

Early diagnosis of tuberculosis and universal drug-susceptibility testing are the first steps necessary to identify the adequate treatment for individual patients and to prevent the spread of disease at the population level. Novel tests that reach "the missing three million patients" and curb the epidemic of drug-resistant tuberculosis are needed. These tests need to have improved performance characteristics and/or reach lower levels of the healthcare system and be affordable as well as link to the needs around new drug/regimen development.

This article describes the efforts that lead to the identification of the highest priority diagnostic needs in tuberculosis and the consensus-building process that resulted in target product profiles (TPPs) for tests to address those needs.

METHODS

Defining a List of Needs

Through interviews with representatives from national tuberculosis programs, clinical experts from industrialized, middle, and low-income countries, researchers, and clinical laboratory experts, a "wish-list" was compiled defining the most important diagnostic needs for tuberculosis [2]. In addition, the literature was searched, and recent reports and position papers were consulted. A list was then assembled of tests needed to fill important gaps in the current diagnostic landscape and whose development would be feasible in the near future [3].

Prioritization Exercise

Once a list of diagnostic needs was developed, a prioritization exercise was done in order to establish a rank order of the tests and to identify those that were perceived of highest priority [3]. Five different predefined expert groups were consulted: patient and community advocates, field practitioners and clinicians, experts from national tuberculosis programs, modelers, and market experts. All experts rated the diagnostic needs based on 10 different criteria having a 5-year time frame for deployment in mind. The criteria that were evaluated included the prioritization for their respective stakeholders group, the potential for scale up of a test addressing the respective need, and the magnitude of the effect of a test on tuberculosis incidence and mortality reduction as well as the market potential for the test.

TPP Development and Refinement

For the highest rated diagnostic needs, comprehensive TPPs were developed by McGill University, Montreal, Canada, and FIND, Geneva, Switzerland. The TPPs were assembled based on a literature search and interviews with experts and then revised in several rounds with the input provided by researchers, clinicians, policy makers, test developers, and funders. As a result of these extended and reiterative consultations, detailed and comprehensive TPPs were developed. In addition, shorter versions including only the most important characteristics were prepared and used for the consensus building process.

Consensus Building

The shortened versions of the TPPs were presented to a large stakeholder audience that included clinicians, implementers, and representatives of countries and national tuberculosis programs in a "Consensus Meeting on high-priority Target Product Profiles" convened in April 2014, by the World Health Organization (WHO) on behalf of the Global Laboratory Initiative and the New Diagnostics Working Group of the Stop TB Partnership. Leading up to the meeting, a Delphi-like process was used to facilitate consensus building. The shortened TPPs were sent to all invited meeting participants (excluding individuals working in industry in order to avoid possible bias). Individuals were asked to rate the level of agreement with each of the proposed characteristics outlined in the TPPs. It was prespecified that consensus would be achieved when at least 50% of the individuals completing the Delphi-survey would agree with the proposed characteristics. Only characteristics for which less than 75% of the responders agreed or a distinct subgroup disagreed were ultimately discussed in the consensus meeting. The final TPPs were published by WHO and partners in October 2014 [4]. This article presents the final TPPs as they were published in the meeting report.

RESULTS

In interviews and reviews of publications, the tuberculosis community identified the need for developing several tuberculosis diagnostic tests in addition to the currently available tools [2]. The list of tests (Table 1) includes triage and screening tests [5], tests for patients difficult to diagnose (ie, children, patients with human immunodeficiency virus [HIV] and patients with extrapulmonary tuberculosis) [6], a simple non-sputum-based biomarker test for diagnosis of active tuberculosis [7], a molecular smear-replacement test [8] at the microscopy center level or at even lower levels of care, drug-susceptibility tests (DST) that could be done in decentralized or centralized settings [9], a biomarker test for diagnosis of a latent tuberculosis infection that predicts progression to active tuberculosis [10] and a test for treatment monitoring [11].

Table 1. Identified Needs for Diagnostic Tests Categorized by Main Indication

TRIAGE, RULE OUT AND SYSTEMATIC SCREENING
Triage test for those seeking care[a]
An HIV/ART clinic-based test to rule out active TB
Systematic screening test for active case finding
RAPID TB DIAGNOSIS (WITH OPTIONAL DRUG SUSCEPTIBILITY TESTING)
Rapid, sputum-based, cartridge-based, molecular test for microscopy centers (with the option of add-on DST cartridge)[a]
Rapid biomarker-based instrument-free test for non-sputum samples (which can also detect childhood and extrapulmonary TB)[a]
Multiplexed test for TB and other infectious diseases
NEXT-GENERATION DRUG SUSCEPTIBILITY TEST
Centralized, high-throughput, drug susceptibility test (incorporating new drugs to support the roll out of new TB Rx regimens post 2014)
TREATMENT MONITORING TEST
Treatment monitoring test (test for cure)
PREDICTIVE TEST FOR LATENT TB INFECTION
Predictive test for latent TB infection at high risk of active TB

Abbreviations: ART, antiretroviral therapy; DST, drug-susceptibility tests; HIV, human immunodeficiency virus; TB, tuberculosis.

[a] Highlights the tests that are being addressed in this article. Target product profiles for the other identified needs are being developed independent of the effort described herein by FIND and partners.

The priority-setting exercise ultimately identified the following tests as the key priorities, which would have the most impact on incidence and morbidity reduction and potential for market entry and scale up over the coming 5 years [3].

1. A rapid sputum-based test as a replacement for smear-microscopy ("smear-replacement test") with or without DST;
2. A rapid non-sputum-based test capable of detecting all forms of tuberculosis via the identification of characteristics biomarkers or biosignatures ("non-sputum based biomarker test");
3. A triage test, which should be a simple, low cost test for use by first-contact health care providers as a rule-out test ("triage test");

More details of the priority setting exercise can be found elsewhere [3]. Four TPPs were ultimately developed, dividing up the rapid sputum-based test as a replacement for smear-microscopy into one with a DST component ("rapid DST") and another one ("smear-replacement test"). The 3 TPPs that address tuberculosis detection are presented in this article. The fourth TPP that addresses the "rapid DST" is presented separately (see Denkinger CM et al in this supplement) as it discusses the very complex field of drug susceptibility testing.

TPP for a Smear-replacement Test for Tuberculosis Detection
Rationale
Smear microscopy is the most widely used tuberculosis test in high-burden countries, and its sensitivity limitations are well known [12]. The sensitivity of newer rapid tools for tuberculosis detection (eg, Xpert) still does not reach that of culture [13, 14]. More sensitive tests are needed so that patients with tuberculosis can be identified upon first presentation to the health care system and so that patients with paucibacillary disease (eg, HIV patients and children) are detected.

Xpert MTB/RIF ("Xpert," Cepheid, Inc., Sunnyvale, California) has enabled more timely and sensitive diagnosis of tuberculosis over smear microscopy and up-front DST for the key drug (rifampin) in the first-line treatment regimen [15–17]. However, the use of Xpert is limited by its cost and infrastructure requirements (eg, power, temperature controlled environment), which prohibits its placement and use in most microscopy centers [18, 19]. The rollout of Xpert has also demonstrated that new diagnostic tools do not necessarily reach additional people eligible for testing or increase the overall number of tuberculosis cases diagnosed, if they are implemented within established care settings (although Xpert does increase the number of bacteriologically confirmed cases) [20]. On the other hand, there is an increasing number of molecular tests in the pipeline that aim to be more sensitive and are specifically designed for use in resource-limited settings such as microscopy centers or peripheral health clinics [21]. Other assays for detection may conceivably be feasible as well (eg, antigen detection), but the molecular pipeline appears to be the most promising in the near future.

TPP characteristics
A more sensitive smear-replacement test would increase the number of patients diagnosed with tuberculosis and might reduce transmission and morbidity through earlier diagnosis and treatment (Table 2) [22]. Ideally a test would aim for a better sensitivity than Xpert for tuberculosis detection and be as good as liquid culture (ie, diagnostic sensitivity of >95% in comparison to culture; analytical sensitivity of less than 4.5 genome equivalents/reaction and <10e2 CFU/assay on one sample). Such a test could obviate further need for culture in drug-susceptible tuberculosis and potentially improve the trust of clinicians and patients in the diagnostic performance of tests and thereby reduce empiric treatment and overtreatment [24].

Modeling work has demonstrated that even a test with performance characteristics better than smear (50% detection of smear-negative) yet inferior to Xpert, if employed at microscopy centers and combined with good linkage to treatment, would result in a reduction in transmission over deployment of Xpert at a district level [22]. Whether up-front resistance testing such as detection of rifampin resistance in Xpert is beneficial will depend on the local epidemiology of drug-resistance and the trade-offs made by including DST (eg, in respect to time to result). A test at the level of a microscopy center would also leverage the existing treatment infrastructure for drug-susceptible tuberculosis that is already in place in these settings. Furthermore, if a smear-replacement test can also be used for treatment monitoring (eg, through detecting viable bacteria), it would be able to completely replace smear microscopy and would be more likely to be adopted by tuberculosis programs.

A sputum-smear replacement test should ideally have a fast turn-around time and allow for batching as well as random access to rapidly inform a treatment decision at the time of the first visit and link to further care [25, 26]. Due to conditions that prevail in microscopy centers in high-burden countries, a robust test with very simple sample preparation and minimal operational requirements will be necessary [8, 18]. Minimal sample handling (ie, total hands-on steps after obtaining sample) and no precision volume control and precision time steps should be required to ensure that the test is feasible with the level of expertise and training that can be expected at microscopy centers [8, 18].

Continuous power is not always available at microscopy centers in high tuberculosis burden countries; therefore, a battery operated device with charge possibility (conceivably through solar power) would be most ideal in order for a test to fit the entire breadth of settings in microscopy centers [8, 18]. High environmental temperatures and high humidity (up to 50°C and 90% humidity) are often a problem in countries where tuberculosis is endemic. Dusty environments are common and adequate protection of optics and moving parts should be considered [27]. Maintenance and calibration require special attention to ensure functionality of equipment particularly at peripheral centers. The average time to equipment/module failure should ideally be more

Table 2. TPP for a Smear-replacement Test for Tuberculosis Detection

Characteristic	Optimal Requirements	Minimal Requirements
Scope		
Goal	To develop a sputum-based test for detecting pulmonary TB at the microscopy-center level of the health-care system to support the initiation of TB therapy during the same clinical encounter or the same day	
Target population	Target groups are all patients suspected of having pulmonary TB who are able to produce sputum, in countries with a medium prevalence to a high prevalence of TB as defined by WHO[a]	
Target user of the test[b]	Health-care workers with a minimum amount of training (that is, with skills that are similar to or less demanding than those needed for performing smear microscopy)	
Setting (level of the health-care system)	Microscopy-center level (primary health-care centers with attached peripheral laboratories) or higher levels of the health-care system	
PERFORMANCE CHARACTERISTICS		
Diagnostic sensitivity[b]	Sensitivity should be >95% for a single test when compared with culture (for smear-negative cases it should be >68%; for smear-positive it should be 99%)	Sensitivity should be >80% for a single test when compared with culture (for smear-negative cases it should be >60%; for smear-positive it should be 99%)
Diagnostic specificity[b]	>98% specificity when compared with culture	
Possibility of using test for treatment monitoring	Yes: a test that is able to replace smear microscopy and also be used to monitor treatment is more likely to be adopted and more likely to completely replace smear microscopy	No
OPERATIONAL CHARACTERISTICS		
Manual preparation of samples (steps needed after obtaining sample)	No steps or 1 step; precise volume control and precise timing should not be required	A maximum of 2 steps; precise volume control and precise timing should not be required
Reagent integration	All reagents should be contained in a single device	A maximum of 2 external reagents should be required; these should be part of test kit
Data export (connectivity and interoperability)	Integrated ability for all data to be exported (including data on use of the device, error rates and rates of invalid tests, and personalized, protected results) over a USB port and network	Integrated ability for all data to be exported (including data on use of the device, error rates and rates of invalid tests, and non-personalized results) over a USB port
Time to result[b]	<20 min	<2 h
Power requirements	Battery operated with recharging capability and a circuit protector	
Maintenance and calibration[b]	Preventative maintenance and calibration should not be needed until after 2 y or 5000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely or no calibration should be required	Preventative maintenance should not be needed until after 1 y or 1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely, should calibrate itself or no calibration should be required
Operating temperature and humidity level	Between +5°C and +50°C with 90% humidity	Between +5°C and +40°C with 70% humidity
Reagent kit – storage, stability, and stability during transport	2 years at 0°C to +50°C with 90% humidity; should be able to tolerate stress during transport (72 h at +50°C); no cold chain should be required	12 months at 0°C to +40°C with 70% humidity; should be able to tolerate stress during transport (72 h at +50°C); no cold chain should be required
Internal quality control	Full internal process controls are necessary, including controls for sample processing and amplification (for NAAT)	
PRICING		
Price of individual test[b] (costs of reagent only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US$ 4.00 for detecting TB	<US$ 6.00 for detecting TB
Capital costs for instrument[b]	<US$ 500 per module	<US$ 1400 per module

Adapted with permission from WHO consensus meeting report on TPPs [4]

Abbreviations: NAAT, nucleic acid amplification test; TB, tuberculosis; TPPs, target product profiles; WHO, World Health Organization.

[a] High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population [23].

[b] These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey.

than 2 years, and a maintenance alert should indicate the need for preventative maintenance as it is unlikely that the same person will always handle the device and records will be kept on duration of use of a device [28]. Only simple tools and minimal expertise should be required for maintenance and repair of the equipment given the difficulty of service visits in peripheral settings [8, 18]. The scale at which a new test is adopted will depend substantially on how well it meets the specified operational characteristics.

TPP for a Non-sputum-based Biomarker Test for Tuberculosis Detection
Rationale
A highly sensitive test based on a biological sample other than sputum (such as urine, blood, saliva, or exhaled air) suitable for implementation at lower levels of care would conceivably help shorten the delay before diagnosis and enable early treatment (and thus reduce morbidity, mortality and transmission) [29, 30] (Table 3). A non-sputum based sample could also enable the diagnosis of extrapulmonary tuberculosis (EPTB) and tuberculosis in children as well as the diagnosis in patients presenting in an earlier stage of the disease (eg, patients who do not have a productive cough to provide a sputum) [25, 31].

TPP characteristics
A non-sputum based biomarker test ideally should be at least as accurate as Xpert 98% sensitive for smear-positive, culture-positive pulmonary tuberculosis (PTB), and 68% sensitive for smear-negative, culture-positive PTB in adults; however, any improvement over smear microscopy could be of value if the test has operational characteristics that make it easy to perform and uses a non-sputum-based sample [32]. For children, a test sensitivity equal or better than 66% for intrathoracic tuberculosis and equal or better than 80% for extrapulmonary tuberculosis (EPTB) in adults would be optimal, as this can currently be achieved on the appropriate samples with Xpert [1, 6, 33]. Similar to Xpert, the specificity of the test should at least be 98% compared against a microbiological reference standard. Ideally the test should be suitable for use at lower levels of the health-care system where it can reach more patients, and it should ideally not require laboratory facilities [22, 34]. Given the deployment at lower levels of the health-care system, an instrument free test would be ideal, but a small (eg, handheld) device is acceptable and would conceivably add benefits (eg, connectivity). The operational characteristics defined for a smear-replacement test at the microscopy center need to be met at a minimum.

TPP for a Community-based Triage/Referral Test for Identification of Tuberculosis Suspects
Rationale
Most individuals who present themselves to health facilities with symptoms suggestive of tuberculosis do not have tuberculosis. In order to rule out tuberculosis quickly a low-cost triage test is necessary. Only triage test positive patients will then require confirmatory testing [5, 35] (Table 4).

TPP characteristics
A triage test needs to be a simple, low-cost test with high sensitivity for use by first-contact providers in the community (eg, community health workers). Such a test can rule out tuberculosis when the result is negative. Individuals with a positive result are directed to further evaluation with a confirmatory test (eg, Xpert). Sensitivity of a triage test should ideally be as good as that of the confirmatory test (>95% of confirmatory test) as otherwise patients would be missed by the test and the strategy of testing all patients with the confirmatory test would theoretically result in a higher case notification rate. However, if a triage test is done at lower levels of care and is easier to do, conceivably more people suspected of having tuberculosis will be tested. Consequently, the test might increase the number of tuberculosis patients identified even if its sensitivity is lower than that of the confirmatory test. Therefore, the minimal sensitivity in the TPP was defined to be greater than 90% compared to the confirmatory test.

A triage test might also conceivably diagnose EPTB. For confirmatory testing, a molecular test or culture on an aspirate or biopsy would then be necessary (eg, a biopsy for lymph node tuberculosis). The specificity requirement for a triage test needs to consider the tuberculosis prevalence in the population tested, but consensus was reached that it should be optimally at least 80% and minimally at least 70%. The specificity of the test is one of the main drivers of the cost-effectiveness of an implementation strategy. The lower the specificity of the triage test, the higher the number of confirmatory tests necessary and therefore the lower the cost of a triage test needs to be to result in a cost-effective testing strategy [5].

For successful implementation at the community level, a triage test should ideally use an easily accessible sample (eg, urine, finger stick blood). The test should optimally be device-free or if a device is needed it should at least be battery-operated [8, 18]. The ideal time-to-result (including sample preparation and processing time) has not been studied; however, a rapid test is more likely to be integrated within the work flow and result in same visit decision making.

The main characteristics of these TPPs were discussed and agreed upon in the "Consensus Meeting on high-priority Target Product Profiles" convened by the World Health Organization on behalf of the Global Laboratory Initiative and the New Diagnostics Working Group of the Stop TB Partnership in April 2014 and published in October 2014 [4].

DISCUSSION

Novel tests are needed to reach "the missing three million patients" and curb the epidemic of drug-resistant tuberculosis. These tests need to have improved performance characteristics,

Table 3. TPP for a Rapid Non-sputum-based Biomarker Test for Tuberculosis Detection

Characteristic	Optimal Requirements	Minimal Requirements
SCOPE		
Goal	To develop a rapid biomarker-based test that can diagnose pulmonary TB and optimally also extrapulmonary TB using non-sputum samples (for example, urine, blood, oral mucosal transudates, saliva, exhaled air) for the purpose of initiating TB treatment during the same clinical encounter or on the same day	
Target population	Target groups are adults and children including those who are HIV-positive and suspected of having active pulmonary TB or extrapulmonary TB in countries with a medium prevalence to a high prevalence of TB as defined by WHO[a]	
Target user of the test[b]	Health-care workers with a minimum of training	Trained microscopy technicians
Setting (level of the health-care system)	Health posts without attached laboratories (that is, levels below microscopy centers) or higher levels of the health-care system	Primary health-care clinics with attached laboratories; peripheral microscopy centers or higher levels of the health-care system
PERFORMANCE CHARACTERISTICS		
Diagnostic sensitivity for pulmonary TB in adults[b]	Sensitivity should be ≥98% for smear-positive culture-positive pulmonary TB, and ≥68% for smear-negative culture-positive pulmonary TB in adults (that is, sensitivity should be similar to that of the Xpert MTB/RIF assay) Overall pooled sensitivity should be ≥80% in adults with HIV infection	Overall sensitivity should be ≥65% but should be >98% among patients with smear-positive culture-positive pulmonary TB (that is, sensitivity should be similar to that of smear microscopy) Overall pooled sensitivity should be better than the sensitivity of smear microscopy in adults with HIV infection
Diagnostic sensitivity for extrapulmonary TB in adults	Ideally, sensitivity should be ≥80% for all forms of microbiologically confirmed extrapulmonary TB[c,d]	Diagnosis of extrapulmonary TB is an important need, and a test that can diagnose extrapulmonary TB in addition to pulmonary TB will have significant benefits for individual patients; additionally, it is likely to be better accepted in the community of care providers. No lower range of sensitivity was defined
Diagnostic sensitivity in children	Sensitivity for childhood intrathoracic TB should be ≥66% for microbiologically confirmed TB (that is, similar to the sensitivity of the Xpert MTB/RIF assay)[e]	Diagnosis of childhood TB is an important need, and a test that improves the diagnosis of TB in children will have significant benefits for individual patients; additionally, it is likely to be better accepted in the community of care providers. No lower range of sensitivity was defined
Diagnostic specificity[b]	At least as specific as the Xpert MTB/RIF assay for detecting pulmonary TB, extrapulmonary TB and childhood TB (that is, the test should have 98% specificity when compared against a microbiological reference standard); the test should distinguish between active TB and latent or past infection	
OPERATIONAL CHARACTERISTICS		
Sample type	Not invasive or minimally invasive, non-sputum samples (such as, urine, blood, oral transudates, saliva, exhaled air)	
Manual preparation of samples (steps needed after obtaining sample)	Sample preparation should be integrated or manual preparation should not be required	A limited number of steps only; precise measuring should not be needed for any step (such as precise measuring of volumes or time)
Time to result[b]	<20 min including time spent preparing the sample	<1 h including time spent preparing the sample
Instrument and power requirement	No instrument needed	Small, portable or hand-held instrument (weighing <1 kg) that can operate on battery or solar power in places where power supplies may be interrupted
Maintenance and calibration[b]	Disposable, no maintenance required	Preventative maintenance should not be needed until after 1 y or >1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the instrument should be able to be calibrated remotely or no calibration should be needed
Operating temperature and humidity level	Between +5°C and +50°C with 90% humidity	Between +5°C and +40°C with 70% humidity

Table 3 continued.

Characteristic	Optimal Requirements	Minimal Requirements
Result capturing, documentation, data display	An instrument-free test with the ability to save results using a separate, attachable reader	The test menu must be simple to navigate; the instrument should have an integrated LCD screen, simple keypad or touch screen, and the ability to save results using either the instrument or a separate reader
Internal quality control	Internal controls should be included for processing the sample and detecting TB	Internal control included only for processing the sample
PRICING		
Price of individual test[b] (costs of reagents and consumables only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US$ 4.00	<US$ 6.00

Adapted with permission from WHO consensus meeting report on TPPs [4].

Abbreviations: HIV, human immunodeficiency virus; LCD, liquid crystal display; TB, tuberculosis; TPPS, target product profiles; WHO, World Health Organization.

[a] High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population [23].

[b] These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey.

[c] The sensitivity for detecting extrapulmonary tuberculosis should also be tested against a composite reference standard that includes culture with or without a nucleic acid amplification test, histology, smear microscopy, biochemical testing, presenting signs, and response to treatment with anti-tuberculosis therapy, depending on site of infection. Xpert MTB/RIF testing has an estimated sensitivity for diagnosing tuberculosis of 84% for lymph node aspirates or other tissue samples, and 55% sensitivity for samples of cerebrospinal fluid, when compared with a composite reference standard, but Xpert MTB/RIF testing requires invasive samples [1].

[d] Xpert MTB/RIF has an estimated sensitivity for microbiologically confirmed tuberculosis of 85% for detecting tuberculosis in lymph node aspirates or other tissue samples, 80% for cerebrospinal fluid, and 44% for pleural fluid but testing requires invasive samples (from aspiration, biopsy, lumbar puncture or thoracentesis).

[e] The test's sensitivity in children should be evaluated against a composite reference standard as defined by an international panel of experts [6].

reach lower levels of the health-care system and reduce cost of diagnostic algorithms as well as link to the needs around new drug/regimen development. TPPs are important to specify end-users needs and target specifications for performance and operational characteristics that product developers should meet. While the TPPs outlined here are all based on a large number of interviews, discussions and extensive literature consultation, still many of the characteristics rely on assumptions and the consensus of expert opinion. Also, the TPPs specify the needs across a wide spectrum of settings with substantial potential differences. While modeling might be of benefit in this context, the understanding of the most essential parameters, particularly for tests that would reach a patient population that is currently not reached by tests (eg, triage test), is limited and modeling outputs are often restricted to defining the key drivers of impact and setting boundaries for those characteristics in sensitivity analyses [5, 36]. As further data become available from operational research and modeling, the outlined TPPs may require refinement. Particularly, defining the acceptable costs is difficult and transparent discussions around diagnostic pricing, cost structure and hidden costs on the one hand and affordability and cost-effectiveness on the other hand are necessary.

New tuberculosis diagnostic tests able to improve tuberculosis detection for EPTB, tuberculosis in children and other forms of paucibacillary tuberculosis could be of great benefit for individual patient management [33, 37, 38]. An outstanding question in this context concerns which reference standard should be considered to assess test accuracy for the diagnosis of these forms of the disease. Indeed microbiological culture, commonly used as the reference standard for establishing a definitive diagnosis of tuberculosis, performs poorly in children and EPTB patients [39–41]. Therefore, test accuracy for the detection of EPTB and tuberculosis in children should be evaluated against a composite reference standard including multiple diagnostic methodologies as well as clinical diagnosis criteria. A composite reference standard for the evaluation of diagnostics for childhood tuberculosis has been defined by an international expert panel and is currently being updated and revised based on latest available evidence [6, 42].

Furthermore, the development of TPPs only represents a first step to address test developers' needs. The next question that needs to be addressed is the current and potential volume and market for the new tests. This is a key issue for test developers as they consider an investment in this field [43]. To estimate the potential market, one first has to assess the currently served market. The last large-scale market assessment for tuberculosis diagnostics was performed by FIND and TDR in 2006 [44]. More recently market assessments were done for 4 BRICS countries (Brazil, South Africa, China, and India) under the lead of McGill University in collaboration with FIND, UNITAID, the New Diagnostics Working Group of the Stop TB Partnership, and multiple country level partners. The work will be documented in separate publications, with the first article published being the market assessment for Brazil [45]. An assessment of

Table 4. TPP for a Community-based Triage/Referral Test for Identification of TB Suspects

Characteristic	Optimal Requirements	Minimal Requirements
SCOPE		
Goal	To develop a test that can be used during a patient's first encounter with the health-care system to identify patients with **any symptoms of or risk factors for active TB**, including patients coinfected with HIV, those who do not have TB and those who need referral for further confirmatory testing	To develop a test that can be used during a patient's first encounter with the health-care system to identify patients with **any symptoms of or risk factors for active pulmonary TB**, including patients coinfected with HIV, those who do not have TB and those who need referral for further confirmatory testing
Target population	Adults and children with signs and symptoms of **active TB at any site** in countries with a medium prevalence to a high prevalence of TB as defined by WHO[a]	Adults and children with signs and symptoms of **active pulmonary TB** in countries with a medium prevalence to a high prevalence of TB as defined by WHO[a]
Target user of the test[b]	Community health workers and informal providers who have had a minimum of training	Health workers trained to the level of auxiliary nurses
Setting (level of the health-care system)	Community level or village level or higher levels of the health-care system	Health posts and primary-care clinics or higher levels of the health-care system
PERFORMANCE CHARACTERISTICS		
Diagnostic sensitivity[b]	Overall sensitivity should be >95% when compared with the confirmatory test for pulmonary TB;[c] no lower range of sensitivity was defined for extrapulmonary TB[d]	Overall sensitivity should be >90% compared with the confirmatory test for pulmonary TB[c]
Diagnostic specificity[b]	Specificity should be >80% compared with the confirmatory test	Specificity should be >70% compared with the confirmatory test
OPERATIONAL CHARACTERISTICS		
Sample type	Non-sputum samples (such as urine, oral mucosal transudates, saliva, exhaled air or blood from a finger-stick)	Sputum; non-sputum samples are preferred (such as urine, oral mucosal transudates, saliva, exhaled air, or blood from a finger-stick; imaging technology
Manual preparation of samples (steps needed after obtaining sample)	Sample preparation should be integrated or manual preparation should not be required (excluding waste disposal); precise timing and measuring should not be required	2 steps (excluding waste disposal); precise timing and measuring should not be required
Time to result[b]	<5 min	<30 min
Instrument and power requirement	None	Small, portable or hand-held device (weighing <1 kg); should have an option for battery power or solar power
Maintenance and calibration[b]	Disposable, no maintenance required	Preventative maintenance should not be needed until after 1 y or 1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely, should calibrate itself, or no calibration should be required
Operating temperature and humidity level	Between +5°C and +50°C with 90% humidity	Between + 5°C and +40°C with 70% humidity
Result capturing, documentation and data display	An instrument-free test with visual readout and with the ability to save results using a separate, attachable reader	The test menu must be simple to navigate; the instrument should have an integrated LCD screen, a simple keypad or touch screen, and the ability to save results using either the instrument or a separate reader
Internal quality control	Internal controls should be included for processing the sample and detecting TB	Internal control included only for processing the sample
PRICING		
Price of individual test[b] (costs of reagents and consumables only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US$ 1.00	<US$ 2.00

Adapted with permission from WHO consensus meeting report on TPPs [4].

Abbreviations: HIV, human immunodeficiency virus; LCD, liquid crystal display; TB, tuberculosis; TPP, target product profiles; WHO, World Health Organization.

[a] High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population [23].

[b] These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey.

[c] The performance characteristics of the triage test need to match those of the confirmatory test that will be used.

[d] The sensitivity of the triage test should be compared with the sensitivity of a composite reference standard (that includes culture with or without a nucleic acid amplification test, histology, smear microscopy, biochemical testing, presenting signs and response to treatment with anti-tuberculosis therapy, depending on site of infection) to account for the fact that the test may detect cases of early tuberculosis or extrapulmonary tuberculosis in cases in which a standard microbiological reference standard might not perform well.

the market for a potential smear-replacement test has also been published [12]. The market potential for the novel tests described in the TPPs above was assessed based on the served available market combined with country specific epidemiological data. The results of these market projections are presented in this supplement in a separate article (see Kik et al [3, 12]).

The achievable volume for a test will be part of the consideration when test developers define the test price. For countries the question will be whether the rollout of a test is possible given the available budget. This question can be answered by considering the number of patients that will be tested, the likely algorithms with which a test will be used, and the available country budget based on the current spent [46]. The results may on the one hand inform test developers as they consider the price point for a novel test and on the other hand it will inform national programs, donors, and funders. Such an exercise was undertaken considering the 4 novel TPPs and is presented here in a separate article (Pantoja et al [46]).

In summary, this article describes 3 out of 4TPPs that were identified as the highest priority by the tuberculosis community and the consensus that was reached on the most important performance and operational characteristics. Our work, together with complementary work presented in this supplement, aims to facilitate the interaction between the tuberculosis community and the diagnostics industry with the goal of leading the field toward achieving the post-2015 global targets [47].

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Target Product Profile of a Molecular Drug-Susceptibility Test for Use in Microscopy Centers

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Background. Current phenotypic testing for drug resistance in patients with tuberculosis is inadequate primarily with respect to turnaround time. Molecular tests hold the promise of an improved time to diagnosis.

Methods. A target product profile for a molecular drug-susceptibility test (DST) was developed on the basis of a collaborative effort that included opinions gathered from researchers, clinicians, policy makers, and test developers on optimal clinical and operational characteristics in settings of intended use. In addition, the current diagnostic ecosystem and the diagnostic development landscape were mapped.

Results. Molecular DSTs for detecting tuberculosis in microscopy centers should ideally evaluate for resistance to rifampin, fluoroquinolones, isoniazid, and pyrazinamide and enable the selection of the most appropriate treatment regimen. Performance characteristics of DSTs need to be optimized, but compromises can be made that depend on the trade-off between a false-positive result and a false-negative result. The operational requirements of a test will vary depending on the site of implementation. However, the most-important considerations pertain to quality control, maintenance and calibration, and the ability to export data.

Conclusion. This target product profile defines the needs as perceived by the tuberculosis stakeholder community and attempts to provide a means of communication with test developers to ensure that fit-for-purpose DSTs are being developed.

Keywords. tuberculosis; diagnostics; molecular testing; point of care.

Progress has been made in improving tuberculosis cure rates globally, but drug-resistant tuberculosis is threatening that progress in many regions. In a 2014 report, the World Health Organization (WHO) estimated that only 8.5% of new tuberculosis cases and 17% of bacteriologically confirmed cases requiring retreatment received drug resistance testing and that, 480 000 people developed multidrug-resistant (MDR) tuberculosis [1].

While the number of patients with MDR tuberculosis or rifampin resistance detected worldwide increased between 2012 and 2013 by 20%, more than half of the estimated MDR tuberculosis cases still remain undiagnosed [1]. The majority of these MDR tuberculosis cases globally are estimated to be among new cases, which is why the global tuberculosis strategy after 2015 calls for universal drug resistance testing [2].

Current phenotypic tests for drug resistance are inadequate primarily with respect to turnaround times and, thus, time to initiation of therapy, which can influence patient outcomes [3]. Molecular tests hold the promise of an improved time to diagnosis, and the Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, California) has demonstrated the benefit of combining both tuberculosis detection and up-front resistance testing for rifampin [4]. Rifampin was chosen as the target for that
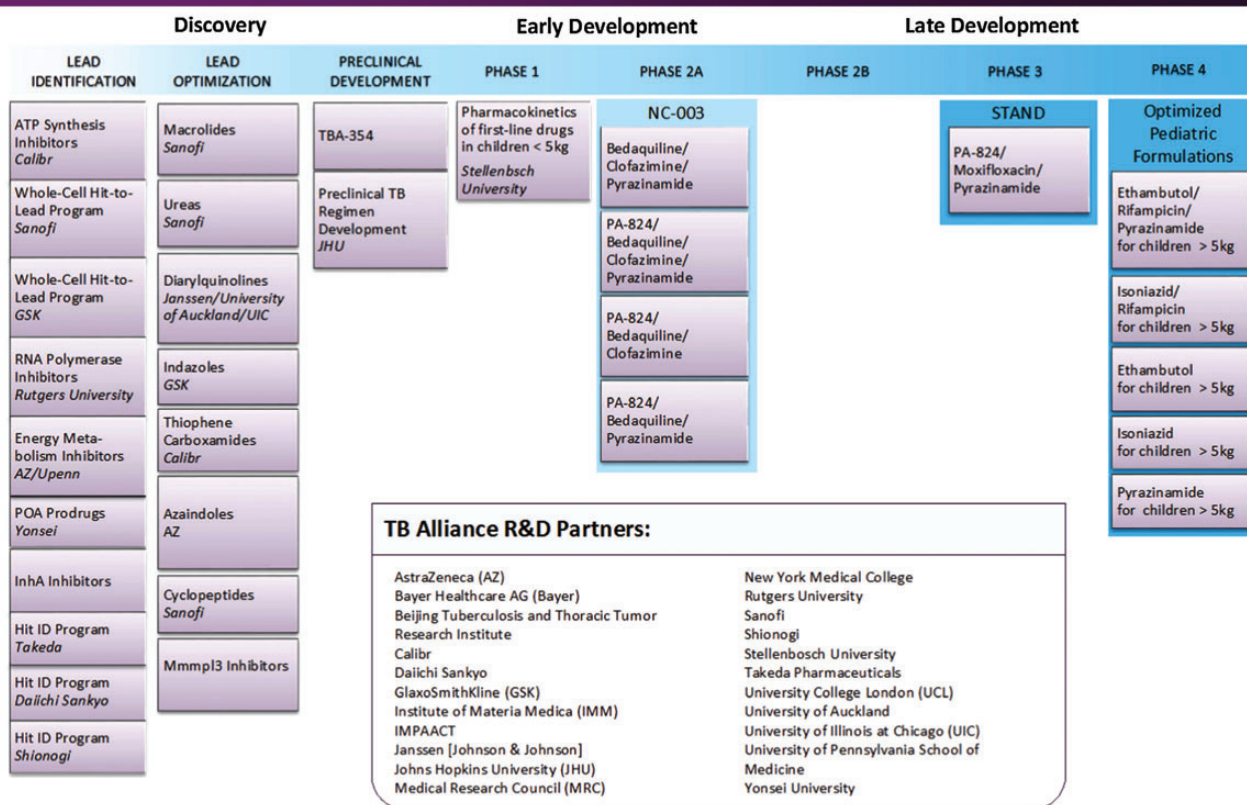
Figure 1. Tuberculosis Alliance pipeline. Reproduced with permission of the TB Alliance [13].

assay because patients with rifampin-resistant tuberculosis require treatment with second-line antituberculosis drugs [5]. A number of other molecular tests are now in the pipeline, with some aiming for an increased drug resistance testing portfolio [6]. Several novel molecular tests are being developed for the peripheral laboratory setting, as opposed to the centralized, referral laboratory [7].

A tuberculosis test that provides results in <2 hours can enable a decision on which regimen to choose or a referral decision at the time of the patient's first visit to a tuberculosis treatment center (ie, at the point of care) [8, 9]. This is especially relevant over the coming years as novel alternative first regimens are emerging [10, 11]. Currently, there is only 1 first-line regimen, which includes isoniazid, rifampin, pyrazinamide, and ethambutol (HRZE). An alternative regimen evaluated for first-line therapy, REMox (rifampin, moxifloxacin, pyrazinamide, and ethambutol or isoniazid), was recently shown to be inferior to HRZE in a phase 3 clinical study [12], but other fluoroquinolone-based regimens are being explored [13]. Figure 1 shows the current tuberculosis drug pipeline. PaMZ (Pa824,

moxifloxacin, and pyrazinamide) was shown to be effective in a phase 2b trial [14] and will be evaluated in a phase 3 trial, which started in November 2014. If the phase 3 study shows this regimen to be beneficial, it could be implemented over the coming years (planned start, 2018) as an alternative to the standard regimen.

A detailed, consensus-based target product profile (TPP) is necessary to align new tuberculosis diagnostic test development with new tuberculosis drug regimens and outline the characteristics of resistance testing that would meet medical and public health needs at the level of the microscopy center, to inform test developers [15].

METHODS

The development of the TPP described here was a collaborative effort that included opinions from researchers, clinicians, policy makers (global and national), and test developers. First, we mapped the current diagnostic ecosystem to understand which diagnostic tests are used in disease-endemic countries and

specific healthcare settings. This was based on observations from national tuberculosis programs and surveys [16]. In addition, market analyses in emerging economies (data for Brazil only have been published to date; data for South Africa, India, and China are to follow) [17] and a literature search of operational research on tuberculosis drug resistance testing were performed. Second, >200 researchers in the field and clinicians, as well as clinical laboratory experts from low-burden and high-burden countries, were surveyed about preferences for the prioritization of drug resistance testing, considering currently available and novel regimens (ie, PaMZ and other fluoroquinolone-based regimens), interpretation and use of results with suboptimal performance characteristics, and other related questions (Daniela Cirillo and Martina Casenghi, personal communication, 2014). In addition, mathematical models were used where available to support decision making around optimal test characteristics [18–20].

Third, a landscaping exercise was performed to create a knowledge base of available molecular platform technologies and molecular assays that could detect tuberculosis and different resistance targets (FIND, unpublished internal data). This was critical to inform the feasibility of achieving target specification within the expected time frame of development (eg, what can be realistically achieved in terms of performance given a 5-year timeline). Key inputs for this exercise were gathered from literature searches, a survey and discussions with the diagnostics industry and academic groups at trade shows and other venues.

To gain a better understanding of the necessary operational characteristics of the proposed diagnostic test, a survey was conducted of the conditions present in microscopy centers of tuberculosis-endemic countries [21]. Data on the number of microscopy centers and average number of tests performed per center were gathered from publications (Demographic and Health Surveys Project; http://www.measure dhs.com) [22]. Needs associated with throughput, times to results, and results documentation were obtained from clinician and laboratory experts in the field. Expert advice was also obtained to inform specifications around data export and connectivity of the diagnostic test (to enable eHealth and mhealth solutions).

Data to inform the specific price range (ie, the lowest preferred and highest acceptable/affordable cost) for a diagnostic test were difficult to obtain. Ideally, the question of cost should be addressed from several perspectives: What are the costs to the test developers for development and production of a novel test? What is the potential market of a test? What would be a range of pricing that would make the test cost-effective (ie, the cost would be justified by the gain in improved health outcomes and the costs averted with the test, eg, shortened therapy or infection control)? What price of the test would be affordable to high-burden countries, considering their currently available

budget for tuberculosis diagnosis? Work is currently ongoing to inform these estimates. A summary of an affordability analysis performed by Pantoja et al is presented as part of this Supplement.

The original draft of the TPP was assembled by FIND with input from all authors. Subsequently, it underwent several rounds of revision, including contributions from the Working Group on Assay Development in the Diagnostic Forum, managed by the Critical Path to Tuberculosis Drug Regimens (CPTR). A shortened version of the TPP was presented to a large stakeholder audience that included >50 clinicians, implementers, and representatives of countries and national tuberculosis programs in a meeting on high-priority target product profiles convened in April 2014 by the WHO on behalf of the Global Laboratory Initiative and the New Diagnostics Working Group of the Stop TB Partnership. The final TPP was published by the WHO and partners in October 2014 [23]. This article discusses the final TPP.

RESULTS

A TPP was compiled using a test developers' perspective with the assumption that new first-line treatment regimens will be implemented and available, at least initially, in parallel to current standard-of-care regimens. We subdivided the TPP by scope, pricing, performance, and operational characteristics (Tables 1–3). Each characteristic refers to a specific requirement or specification that is measurable. For each characteristics, a minimal and optimal specification was defined. The minimal specification for a specific characteristic refers to the lowest acceptable specification for that characteristic (although a test may still be acceptable if shortcomings are only missed marginally and are counterbalanced by other advantages). The optimal specification for a specific characteristic provides the ideal value for that characteristic. Meeting the optimal characteristics provides the greatest differentiation from existing methods and the greatest influence for the end users, clinicians and patients. Developers would ideally design and develop their solutions to meet the optimal specification in all characteristics. The optimal and minimal specifications for each characteristic define a range. The characteristics were specified with a development timeline of <5 years in mind.

Scope of Use for the Test

The goal of the assay defined in the TPP is to detect *Mycobacterium tuberculosis* and antituberculosis drug resistance near the point at which case detection and/or treatment initiation would normally occur (eg, microscopy centers and treatment centers; Table 1). Information gained by testing would inform decision making concerning current first-line regimen selection (HRZE, which will likely be available for the foreseeable future), as well as novel regimens (such as PaMZ or other likely

Table 1. Scope of Drug-Susceptibility Tests (DSTs) at Microscopy Centers

DST Characteristic	Optimal/Minimal	References
Goal	Diagnosis of tuberculosis and detection of drug resistance, to inform decision making about the optimal first-line regimen (HRZE, PaMZ, or other fluoroquinolone-based regimens) for treatment and, possibly, to detect the presence of additional resistance to second-line antituberculosis agents and the need for further testing	. . .
Target population	Target groups are all patients suspected of having tuberculosis, with a special focus on those at high risk of morbidity and mortality from drug-resistant tuberculosis, such as people living with HIV and those at high risk of having MDR tuberculosis (eg, household contacts of patients in whom MDR tuberculosis has been diagnosed and persons with a history of tuberculosis, especially those for whom first-line therapy has failed) in countries with a medium prevalence to a high prevalence of tuberculosis, as defined by the WHO[a]	[1, 24]
Target user	Healthcare worker with training necessary for performing smear microscopy	
Lowest setting of implementation (health system level)	Microscopy centers or higher levels of the healthcare system	[21, 25–27]

Adapted with permission from [23].

Abbreviations: HIV, human immunodeficiency virus; HRZE, isoniazid, rifampin, pyrazinamide, ethambutol; MDR, multidrug resistant; PaMZ, Pa824, moxifloxacin, pyrazinamide; WHO, World Health Organization.

[a] High-prevalence countries are those with >40 cases per 100 000 population, medium-prevalence countries are those with 20–40 cases per 100 000 population, and low-prevalence countries are those with <20 cases per 100 000 population [24].

fluoroquinolone-based regimens), and/or the need for further testing for resistance to additional drugs. The target population for testing as defined in the TPP is all patients suspected of having tuberculosis, with a special focus on those at high risk of morbidity and mortality from drug-resistant tuberculosis, such as people living with human immunodeficiency virus (HIV), and those at high risk of having MDR tuberculosis (eg, household contacts of patients with MDR tuberculosis, persons with a history of prior tuberculosis, and persons who did not respond to first-line therapy).

Performance Characteristics

M. tuberculosis Detection

As shown in Table 2, the optimal sensitivity for *M. tuberculosis* detection is higher than currently achieved by Xpert MTB/RIF (>95%; 95% confidence interval [CI], 90%–100%) when using a single test, compared with 2 liquid cultures (smear negative, >68%; smear positive, >99%) [51]. The optimal sensitivity translates into a limit of detection of $<10^2$ colony-forming units/assay in 1 sample. The minimal sensitivity of the test should be >80% (95% CI, 70%–90%), with retained high sensitivity in smear-positive patients (smear positive, 99%) and a smear-negative sensitivity of >60%. We set test specificity to allow use in the population of all patients who might be suspected of having tuberculosis. The specificity should be >98% for a single test, compared with the optimal culture technique for the specific drug tested. No cross-reactivity with other organisms, including nontuberculous mycobacteria, is allowable. Multiplexing capability and the ability to use the platform for different tests (eg, HIV load testing) were judged as valuable features. Although not achievable with existing molecular tests, a test should also be suitable for treatment monitoring, to fully replace smear microscopy.

Resistance Testing

Testing for rifampin, fluoroquinolones (including moxifloxacin), isoniazid, and pyrazinamide resistance was identified as most useful for regimen selection in the near future (Table 2). The TPP prioritized testing for drugs for which resistance-causing mutations have been identified and are known to be of clinically relevant frequency and in which resistance has ≥1 of the following 3 consequences: it seriously affects treatment efficacy, increases the risk of resistance amplification, or strongly predicts resistance to other drugs. Fluoroquinolones and pyrazinamide resistance testing were included because, even if the clinical trial results for PaMZ are not satisfactory, it is very likely that these drugs will be part of novel regimens [13]. No specification was made with respect to whether testing for resistance to a drug should be included together with *M. tuberculosis* detection or whether it should be in a separate step. This decision will depend on many factors, including which performance characteristics can be reached for a certain drug, what the epidemiology of drug resistance is, and what the trade-off might be for including the drug-susceptibility test together with *M. tuberculosis* detection (eg, in terms of time to diagnosis).

Considerations around specific drugs included were as follows. Rifampin is a key component of HRZE and is also an indicator drug for resistance to additional drugs, particularly pyrazinamide and isoniazid (ie, >90% of rifampin-resistant strains are isoniazid resistant and 30%–90% are pyrazinamide resistant) [30–32, 52]. Fluoroquinolone resistance is less closely associated with rifampin resistance (10%–30% of rifampin-resistant strains are fluoroquinolone resistant) [52]. Moxifloxacin is a key component of PaMZ, and it is a suitable replacement of isoniazid in case of isoniazid monoresistance and, along with

Table 2. Performance Characteristics of Drug-Susceptibility Tests (DSTs)

Characteristic	Optimal	Minimal	Reference(s)
Diagnostic sensitivity for *M. tuberculosis* detection	Should be >95% for a single test, compared with 2 liquid cultures; for smear-negative tuberculosis, it should be >68%; for smear-positive tuberculosis, it should be 99%	Should be >80% for a single test, compared with culture (for smear-negative cases, it should be >60%; for smear-positive cases, it should be 99%)	[19]
Diagnostic specificity for *M. tuberculosis* detection	Should be >98% for a single test, compared with culture	Should be >98% for a single test, compared with culture	[4, 28, 29]
Priority of drugs tested	In order of decreasing importance: (1) RIF, (2) FQs (including MOX) (3) INH and PZA (equally important), and (4) AG/CAP; optimally, all drugs would be included, but as a minimum at least RIF should be included		[1, 30–36]
Diagnostic sensitivity for DST, by reference standard			
Genetic sequencing	Should be >98% for detecting targeted SNPs for resistance to RIF, FQs, PZA, INH, and AG/ CAP, compared with genetic sequencing	Should be >98% for detecting targeted SNPs for resistance to RIF and 95% for detecting targeted SNPs for resistance to FQs, PZA, INH, and AG/ CAP, compared with genetic sequencing	[1, 28, 37–42]
Phenotypic DST	>95% for detecting RIF, FQ, PZA, INH, and AG/CAP resistance in comparison to recommended phenotypic culture reference DST for specific antituberculosis agent	>95% for detecting RIF resistance; >90% for detection of FQ, PZA, INH, and AG resistance in comparison to recommended phenotypic culture reference DST for specific antituberculosis agent	[42, 43]
Diagnostic specificity for DST, using genetic sequencing as the reference standard	Should be ≥98% for any antituberculosis agent for which the test is able to identify resistance		[1, 28, 37–39, 42]
Limit of *M. tuberculosis* detection during resistance testing			
First reaction	Should be better than Xpert MTB/RIF for tuberculosis case detection (ie, <4.5 genome equivalents/reaction and <10^2 CFU/assay, using 1 sample	Should be between smear microscopy and Xpert MTB/RIF for tuberculosis case detection (ie, 10^2–10^5 CFU/assay, using 1 sample)	[4, 29]
Second reaction	Should be no worse than Xpert MTB/RIF for tuberculosis case detection (ie, ≥4.5 genome equivalents/reaction and 131 CFU/mL of sputum)	Should be between smear microscopy and Xpert MTB/RIF for tuberculosis case-detection (ie, 10^2–10^5 CFU/assay, using 1 sample)	[44]
Analytical specificity for *M. tuberculosis* detection	No cross-reactivity with other organisms, including nontuberculous mycobacteria	No cross-reactivity with other organisms, including nontuberculous mycobacteria	. . .
Indeterminate results detection, %	<2	<5	. . .
Reproducibility	Interassay coefficients of variance should be ≤10.0% at the high and low extremes of the assay		. . .
Interfering substances	No interference should be caused by substances known to occur in the human respiratory and pulmonary tracts, including blood that could potentially inhibit PCR, and substances used to treat or alleviate respiratory disease or symptoms		. . .
Assay design	Addition or removal of analytes should not require extensive analytical and clinical reverification and revalidation of the assay		. . .
Treatment-monitoring capability	Yes	No	. . .

Adapted with permission from [23].

Abbreviations: AG, aminoglycoside; CAP, capreomycin; CFU, colony-forming units; FQ, fluoroquinolone; HRZE, isoniazid, rifampin, pyrazinamide, ethambutol; INH, isoniazid; MOX, moxifloxacin; *M. tuberculosis*, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction; PZA, pyrazinamide; RIF, rifampin; SNP, single-nucleotide polymorphism.

other fluoroquinolones, is part of the current regimens for MDR tuberculosis [53].

Pyrazinamide is included in HRZE and PaMZ regimens and is a key component for sterilization of infected sites. The prevalence of fluoroquinolone and pyrazinamide resistance (in the absence of rifampin resistance) is poorly defined but is expected to be <3% in most countries across all patients presenting for testing (Matteo Zignol, WHO, personal communication, 2014), with higher values expected in countries where fluoroquinolones are widely used as antibiotic for other infections (eg, India and Pakistan). With this low prevalence, upfront testing of all patients for fluoroquinolone and pyrazinamide

Table 3. Operational Characteristics of Drug-Susceptibility Tests

Characteristic	Optimal	Minimal	References
Sample type	Sputum raw	Sputum raw	. . .
Acceptable range for sample volume	Any sample from 0.1 mL to 10 mL is acceptable	Any sample from <0.5 mL to 2 mL is acceptable	. . .
Manual sample prep (total hands-on steps after obtaining sample)	No steps or 1 step; precise volume control and precise timing should not be required	Maximum of 2 steps; precise volume control and precise timing should not be required	[21, 25]
Reagent integration	All reagents should be contained in a single device	A maximum of 2 external reagents should be needed and, if required, should be included in the test kit	. . .
Time-to-result	<30 min (for detection and resistance testing)	<2 h (for resistance testing alone)	[45, 46]
Daily throughput per module	>25 tests	>5 tests	. . .
Sample capacity and throughput	Multiple samples should be able to be tested at the same time; random access should be possible	Batching should be possible	. . .
Walkaway operation	These features are required; there should not be a need for operator intervention once the sample has been placed into or on the instrument	No more than 1 step of operator intervention should be needed once the sample has been placed into or on the system	. . .
Biosafety	Should have the same requirements as the Xpert MTB/RIF assay	Should have the same requirements as the Xpert MTB/RIF assay	[21, 25, 47]
Waste disposal			
Solid material	Should require no more than smear microscopy; should have the possibility of recycling some waste	Should require no more than Xpert MTB/RIF	. . .
Infectious material	Should require no more than Xpert MTB/RIF	Should require no more than Xpert MTB/RIF	. . .
Multiuse platform	Yes	None required	. . .
Instrumentation	A single integrated system that is modular to allow throughput to be increased if needed	Up to 2 instruments within the system that are independent of each other	. . .
Power requirements	Battery operated with the ability to run for 1 d on the battery and with recharging capability (which could be solar powered) and a circuit protector	Capable of running on standard electricity plus an uninterrupted power supply unit to enable a cycle to be completed in case of a power outage; a circuit protector should be included; the uninterrupted power supply and circuit protector must be integrated within the system	[21, 25]
Maintenance/calibration	Preventive maintenance should not be needed until after 2 y or >5000 samples; an alert should be included to indicate when maintenance is needed; should be able to be calibrated remotely, or no calibration should be needed	Preventive maintenance should not be needed until after 1 y or 1000 samples; an alert should be included to indicate when maintenance is needed; should be able to be calibrated remotely, or no calibration should be needed	[48, 49]
Data analysis	Data analysis should be integrated into the device; a PC should not be required; exported data should be capable of being analyzed on a separate or networked PC		. . .
Result documentation, data display	An integrated results screen and the ability to save and print results should be included; the device should have a USB port	An integrated results screen and the ability to save results should be included; the device should have a USB port	. . .
Regulatory requirements	Manufacturing of the assay and system should comply with ISO EN 13 485 or higher standards or regulations and with ISO IEC 62 304 Medical Device Data Systems; the manufacturing facility should be certified and authorized for use by a regulatory authority that is a member of the International Medical Device Regulators Forum, formerly known as the Global Harmonization Task Force; the assay must be registered for in vitro diagnostic use		. . .

Table 3 continued.

Characteristic	Optimal	Minimal	References
Data export (connectivity and interoperability)	All data should be able to be exported (including data on use of the device, error rates and rates of invalid tests, and personalized, protected results) over a USB port and network; network connectivity should be available through an Ethernet, Wi-Fi, or GSM/UMTS mobile broadband modem or a combination of these; results should be encoded using a documented standard (such as HL7) and be formatted as JSON text; JSON data should be transmitted through HTTP(S) to a local or remote server as results are generated; results should be stored locally and queued during network interruptions use of the device, error rates or rates of invalid tests, and nonpersonalized results) over a USB port to be sent as a batch when connectivity is restored	Integrated ability for all data to be exported from the device in a user-friendly format (including data on use of the device, error rates or rates of invalid tests, and nonpersonalized results) over a USB port	[21, 25, 50]
Electronics and software	Should be integrated into the instrument	Should be integrated into the instrument	. . .
Operating temperature/humidity	5°C–50°C at 90% humidity	5°C–40°C at 70% humidity	[21, 48]
Reagent kit			
Transport	No cold chain should be required; should be able to tolerate stress during transport for a minimum of 72 h at −15°C to 50°C	No cold chain required; should be able to tolerate stress during transport for a minimum of 72 h at −15°C to 40°C	[21, 25]
Storage and stability	2 y at 5°C–40°C with 90% humidity; should be able to tolerate stress during transport for a minimum of 72 h at 50°C; no cold chain should be required	12 mo at 5°C–35°C with 70% humidity; should be able to tolerate stress during transport for a minimum of 72 h at 50°C; no cold chain should be required	[21, 25, 48]
Supplies not included in kit	None	None	. . .
Internal quality control	Full controls for sample processing, amplification, and detection of *M. tuberculosis* should be included		[48, 49]
Training and education	6 work-hours for staff at the level of a microscopy technician	3 d (or 24 work-hours) for staff at the level of a laboratory technician	. . .

Adapted with permission from [23].

Abbreviations: GSM, Global System for Mobile Communications; *M. tuberculosis*, *Mycobacterium tuberculosis*; PC, personal computer; RIF, rifampin; UMTS, Universal Mobile Telecommunications System; USB, universal serial bus.

resistance would require a highly specific test to avoid high numbers of false-positive results, unless the patients had previously been triaged via the detection of rifampin resistance or unless a false-positive result would have limited adverse impact, owing to the existence of alternative first-line regimens [54].

Isoniazid is a key component of HRZE and the most common source of monoresistance, and it is thus a good candidate for inclusion in resistance testing. However, modeling data (at least for Southeast Asia) show that, on a population level, isoniazid testing has minimal incremental value, compared with testing for rifampin alone, to control MDR and isoniazid resistance [33]. This might change if isoniazid monoresistance increases as more isoniazid preventive therapy is rolled out [34]. Furthermore, the individual benefit of knowing the isoniazid resistance status to guide therapy is indubitable [54].

Ideally, resistance testing should also inform providers on decisions about second-line therapy. For second-line drugs, resistance testing for aminoglycosides and capreomycin, in addition to fluoroquinolones, would be critical to inform treatment selection for patients with extensively drug-resistant tuberculosis (XDR) or (pre-) XDR patients (i.e. resistant to either aminoglycosides or fluoroquinolones). However, if inclusion of these drugs results in an increase in test price or complexity, it may be more cost-effective to test for resistance to aminoglycosides and capreomycin with a separate, lower volume test, rather than bundling it with *M. tuberculosis* detection and resistance testing to first-line drugs.

On the basis of these considerations, the importance of drug resistance testing in near-patient settings was rated as follows, in descending order of importance: rifampin, fluoroquinolones

(including moxifloxacin), isoniazid and pyrazinamide (both of which were considered of equal importance), and aminoglycosides/capreomycin. Unless inclusion of resistance testing for a drug adversely affects test cost or performance, all drugs would be included under optimal conditions.

The sensitivity of a rapid molecular method to detect drug resistance can be judged in comparison to a genotypic (sequencing) or phenotypic (culture-based) method. Optimally, new tests should detect individual single-nucleotide polymorphisms (SNPs) encoding rifampin, fluoroquinolone, pyrazinamide, isoniazid, and aminoglycoside/capreomycin resistance at least 98% of the time, comparison with sequencing. This threshold should be considered minimally acceptable for rifampin only; for the other drugs, the sensitivity for detection of individual SNPs should be ≥95%. With a phenotypic comparator, resistance to any given drug should be detected with ≥95% sensitivity. Minimally, the same specification is maintained for rifampin resistance but decreases to 90% for detection of fluoroquinolones, pyrazinamide, isoniazid, and aminoglycosides/capreomycin [1, 28, 37–42]. Optimal and minimal specificity requirements are identical: ≥98% for any drug resistance testing, compared with either phenotypic resistance testing or the sequencing reference standard [1, 28, 37–39, 42].

Operational Characteristics

Because of conditions that prevail in microscopy centers in high-burden countries, tests used in these centers should be robust with very simple sample preparation and minimal operational requirements (Table 3). The degree to which a test gets adopted will likely depend as much on how well a new product meets the specified operational characteristics as on cost or performance [8, 20].

Power Requirements/Tolerance to Environmental Conditions

Ideally, a test should be battery operated (with a functional life of 24 hours when fully charged) and include a recharging solution (eg, solar) and circuit protector. At a minimum, the platform should be capable of being powered by a standard electrical supply and have a backup with an uninterrupted power supply (UPS) to complete any ongoing testing in case of failure of the AC power supply. The UPS and a circuit protector must be integrated within the system. Tolerance to high temperatures (optimally, up to 50°C) and high humidity (90%) is a key criterion for durability and performance of testing in many tuberculosis-endemic settings (Table 3).

Maintenance, Calibration, and Integrated Controls

Required maintenance should be infrequent (optimally, only every 2 years) with a maintenance alert indicating the need for evaluation. Furthermore, it will be essential that only simple tools and minimal expertise are necessary to do the maintenance,

given that service visits are unlikely to be feasible outside of urban settings [48, 49]. No calibration should be required, or remote calibration should be feasible. Full process control, (ie, specifically controlling for sample processing, amplification, and detection) should be integrated into testing [48, 49].

Time to Result

The need for a rapid turnaround time, the possibility of batching and random access, and the testing of multiple samples at the same time are interrelated in their importance, as all of these will define how many samples can be tested per day and how quickly the patient will receive results [45, 46]. Optimally, the turnaround time should be <30 minutes (for detection and resistance testing); although a minimum of 2 hours for resistance testing alone would be acceptable, ideally, detection of *M. tuberculosis* would be reported more rapidly, to prevent loss to follow-up [45, 46].

Sample Preparation

The requirements for the manual sample preparation (ie, the total number of hands-on steps after obtaining the sample) and the results documentation are important characteristics of a test, considering the expertise of the user at the microscopy center level [21, 25]. Optimally, no manual steps or only 1 step should be necessary (and any steps that require precision volume control or precision time steps should be excluded).

Connectivity/Data Export

Although Internet access is not widely available in the settings of intended use, mobile phone capacity is frequently available, even at microscopy centers [21, 25]. This could be leveraged for patient management, quality control, device and supply chain management, and surveillance [50]. Platforms should, ideally, therefore enable full export of data (on device use, error/invalid rates, and personalized, protected results) over a universal serial bus (USB) port and network. The network connectivity should be through Ethernet, Wi-Fi, and/or Global System for Mobile Communications/Universal Mobile Telecommunications System mobile broadband modem. Results should be encoded using a documented standard (such as HL7). At minimum, the platform should have the integrated ability to fully export data (on device sue, error/invalid rates, and nonpersonalized results) from the device in a user-friendly format over a USB port [21, 25].

Cost

Limited data are available on acceptable cost from the perspectives of developers, national treatment programs, and global funders [55]. A higher price than that of the available technologies (Xpert MTB/RIF and Hain Genotype MTBDRplus are currently available under preferential pricing for approximately $10/test) would be justified only if the new tests bring substantial added value in terms of improved performance, greater

suitability for decentralization, and the number of drugs for which resistance can be detected. Cost-effectiveness modeling work is ongoing. A summary of an affordability analysis performed by Pantoja et al is presented as part of this Supplement. Further discussions on an acceptable cost range are necessary as new technologies become available to understand the cost of goods, development, and manufacturing. As the added value in respect to performance and operational characteristics increases, so too might the acceptable costs (to donors like The Global Fund and countries).

DISCUSSION

Expanded availability of drug-susceptibility testing is needed to improve individual patient level outcomes and, as part of tuberculosis control efforts, to improve management of drug resistance. Because of the slowness and complexity of conventional methods, resistance testing is almost never performed at peripheral centers, and results of such tests would therefore not inform selection of first-line therapy when multiple regimens are available [1]. However, testing in the microscopy center requires that a test meet certain operational characteristics to maintain the performance demonstrated in controlled settings [56, 57]. Resistance testing at peripheral settings needs to be complemented by centralized surveillance and testing to inform individualized therapy.

While great strides have been made to improve the understanding of the needs for detection and resistance testing and the various requirements for test use in different healthcare settings, certain key data gaps remain. To improve our understanding of the distribution of drug resistance, the correlation of resistance between drugs, and the trajectories of resistance development over time, population-level surveillance data for different drugs in different regions is necessary. Rifampin and isoniazid data and trajectories are available over recent years, but the understanding of the prevalence of resistance for other drugs is confined to isolated publications [1, 32, 35]. A surveillance effort by the WHO in 5 countries will shed light on the prevalence of pyrazinamide and fluoroquinolone resistance and the correlation with rifampin resistance. This work is complemented by parallel surveillance work in India and China.

Data are also needed on the correlation of mutations with phenotypic results and clinical outcomes and the association with cross-resistance. Here, the scientific community has to work to increase understanding and inform test developers. Efforts to pool sequencing data from different studies and surveillance projects will be essential to better understand the molecular basis of resistance [58]. A coordinated effort to compile the available data across different geographic regions into a database that contains the appropriate meta-data, is vetted and quality controlled, and is readily accessible to all stakeholders

is being initiated by FIND, the New Diagnostics Working Group, and the CPTR [59]. Monitoring of resistance for new drugs (eg, bedaquiline and delamanid) and integration into molecular drug-susceptibility testing should also be considered as they become more widely used.

Further implementation research is necessary to better understand barriers to diagnosis and treatment, as well as overtreatment. What is necessary to ensure that test results lead to earlier treatment and minimize loss to follow-up? Data from the phase 3 drug trials and postintroduction surveillance will further guide the understanding of trade-offs of incorrectly identifying sensitivity or resistance (eg, what percentage of patients would acquire resistance to moxifloxacin and Pa-824 if a test failed to identify pyrazinamide resistance and the patient was only treated with 2 effective drugs?).

This ongoing work will aid the refinement of the specifications outlined in the TPP, making it a dynamic tool for communication with investors, partners, and stakeholders and a tool for tracking results toward appropriate assays for testing drug susceptibility in tuberculosis.

Notes

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Integration of Published Information Into a Resistance-Associated Mutation Database for *Mycobacterium tuberculosis*

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Tuberculosis remains a major global public health challenge. Although incidence is decreasing, the proportion of drug-resistant cases is increasing. Technical and operational complexities prevent *Mycobacterium tuberculosis* drug susceptibility phenotyping in the vast majority of new and retreatment cases. The advent of molecular technologies provides an opportunity to obtain results rapidly as compared to phenotypic culture. However, correlations between genetic mutations and resistance to multiple drugs have not been systematically evaluated. Molecular testing of *M. tuberculosis* sampled from a typical patient continues to provide a partial picture of drug resistance. A database of phenotypic and genotypic testing results, especially where prospectively collected, could document statistically significant associations and may reveal new, predictive molecular patterns. We examine the feasibility of integrating existing molecular and phenotypic drug susceptibility data to identify associations observed across multiple studies and demonstrate potential for well-integrated *M. tuberculosis* mutation data to reveal actionable findings.

Keywords. tuberculosis; drug resistance; resistance-associated mutations; genomic sequencing; drug susceptibility testing; database.

Since 2002 there has been a gradual 1.3% annual decrease in the incidence of tuberculosis worldwide. Although this trend is encouraging, it is too weak to lead to elimination of tuberculosis as a public health problem by 2050, which is the goal of the World Health Organization (WHO) [http://www.who.int/tb/strategy/stop_tb_strategy/en/]. The challenge to stop tuberculosis is severely complicated by the increasing incidence of drug-resistant tuberculosis. Although rapid and accurate detection of tuberculosis will be a key factor in conquering tuberculosis, both treatment of the disease and better success preventing its transmission are significantly boosted by accurate information on drug susceptibility [1]. The WHO defines multidrug-resistant tuberculosis (MDR-TB) as resistance to isoniazid and rifampicin, with or without resistance to other first-line drugs [http://www.who.int/tb/challenges/mdr/tdrfaqs/en/]

With the introduction of new drug combinations and regimens, and patients with potentially more complex resistance profiles, it is imperative to be able to provide a comprehensive profile of drug susceptibility in order to select the correct therapies. Bacterial culture-based drug susceptibility testing (DST) is current "gold standard," but is technically difficult and time-consuming. DST methods are not standardized and results may vary depending on culture techniques employed [2–4], which is especially true for isolates with low-level resistance or when testing resistance to certain second-line drugs. Phenotypic DST methods can also expose laboratory workers to potential infection. Thus new approaches to determining drug resistance are needed.

Detection of resistance-conferring mutations with methods such as polymerase chain reaction and hybridization, targeted sequencing of specific genes, or whole genome sequencing are attractive and promising alternatives to phenotypic DST methods. The data from sequencing especially have been encouraging, with the identification of genes and intergenic noncoding regions associated with drug resistance. However, no systematic study has been performed to correlate genotypic output of either a targeted or whole genome sequencing approach with phenotypic drug response in culture and clinical outcome. In addition, there remains a need to inform logistical decisions on developing simple, rapid, affordable molecular tuberculosis drug-resistance diagnostics, particularly in light of challenges faced by clinical laboratories in low- to middle-resource settings. Currently available DNA sequencers all have reproducibility and performance issues, technical biases, and provide data that require informatics-intensive activities. While welcomed in a research setting, sequencing protocols provide data that need to be reduced to actionable knowledge and may contain false calls (errors) that require base-by-base review. Before sequencing technologies can impact tuberculosis on a global scale, we need to learn how to translate data into statements on drug resistance in a well-supported and reproducible fashion.

To affect guidance in the treatment of patients with tuberculosis, all current analysis and modelling point to the necessity for a solution based on sequence-level results generated as near to the patient as possible [1, 5]. This need for technology proximal to the point of care must be balanced with requirements for data quality. There are multiple sequencing instruments, multiple sample-processing approaches, multiple options for user interfaces, and as-yet incomplete global data associating specific mutations with degrees of resistance to different drugs. Both a protocol to measure mutations and algorithms to interpret the drug resistance implied by mutation data will need to be developed.

Although new sequencing methods are providing an avalanche of genomic information at continually lower cost [6–8], this wealth of information is currently underexploited for diagnostics. Although there are regional and research activities using raw sequencing results from various platforms, there is no generally accepted sequence data-handling approach that is vetted, quality controlled, and readily accessible to the scientific community, let alone usable globally in a clinically constructive way. Efforts to exploit sequencing technologies for tuberculosis treatment and control will require development of solutions tailored to translate this information into easy-to-use and intuitive diagnostic devices.

Data on mutations identified in drug-resistant isolates alone is not enough to determine the association of the mutation with resistance or to demonstrate causality. Therefore, we should focus on data incorporating both susceptible and resistant samples.

Currently, mutation and drug-resistance data for *M. tuberculosis* are scattered across multiple independent databases, journal articles, and their Supplementary Materials. We sought to identify data on isolates appropriate to integrate into a single database to enable querying data across study sources. The purpose of this work was to determine (i) the challenges to integrating mutation and DST data from multiple sources, and (ii) if data, once integrated, allow for systematic analysis that could inform diagnostics development.

METHODS

Published articles and online repositories were identified and reviewed for data on drug resistance-associated single nucleotide polymorphisms. The following criteria were used to prioritize the data sets for inclusion in this integration project:

(i) DST and mutation data on individual isolates preferred over summary statistics (desired, not required), (ii) number of isolates reported (desired more than 50 isolates in a publication), (iii) mutation data on susceptible isolates (required), (iv) easily understandable methods and results (required), (v) DST data on wild-type isolates (required), (vi) drug-level DST results rather than isolate classification solely by MDR or XDR criteria (required), (vii) publications post-2009 (required since data from pre-2010 publications were included via integration of the tuberculosis drug resistance mutation database (TBDReaMDB) [9]), (viii) understandable, well documented, and readily available data tables in articles, Supplementary Tables, or website portals (required), and (ix) publication not on hold or retracted (required).

Table 1 documents the article sources that were included [10–15]. Table 2 documents the online database resources.

Data Integration
The tuberculosis drug resistance database (TBDR) was established to integrate drug resistance mutation data from the different sources and to enable querying for those mutations that have supporting evidence from multiple studies. The intent was to capture as much information as possible from each study yet also allow analysis across diverse studies. During the development of the database specific issues were identified, and flexibility was built into the database. For instance, some studies reported mutations only at the amino acid level while others reported codon changes. Trivial conversions were implemented, such as translating codons to amino acids or nucleotides to the negative strand if appropriate. The database structure enabled on-the-fly summarization of data at the nucleotide level (eg, "S315T (AGC/ACC)"), the most granular level we store, or at the amino acid level (eg, "S315T"). Some source information, such as the genomic coordinates reported by the Broad/GTBDR database, was captured to permit future efforts to bring identical mutations together.

Table 1. Articles Used as Data Sources

PMID	Compact Citation	Comments
21 300 839	Campbell et al, 2011	314 clinical isolates with varied resistance patterns[a]
22 294 518	Casali et al, 2012	1000 sequenced isolates, multiple DST results
23 019 190	Nosova et al, 2013	68 strains were selected at random (38 strains resistant, 30 susceptible to OFX)
24 353 002	Rodwell et al, 2014	Tables 3, 4, 5, 6*A*, 6*B*, and 6*C* provide tabulated results for mutations and DST
24 478 476	Lin et al, 2014	Table 2 summarizes mutations and DST
25 336 456	Miotto et al, 2014	1066 isolates not in Casali et al, 2012, profiled for pncA mutations and PZA DST[b]

Abbreviations: DST, drug susceptibility testing; PMID, PubMed identifier.

[a] Isolate level data communicated by author Dr Posey.

[b] Communicated by authors Drs Cirillo and Miotto.

Some researchers reported mutation observations at the isolate level, while most authors reported just tabulated findings. Sometimes these tabulated findings are co-occurring mutations, which could give some insight into isolate-level observations. TBDR captured as much structure from each study as possible. For isolate-level reports TBDR stored the information on individual isolates then automatically summarized the tabulated results for each drug. For co-occurring mutations, the structure of the data was preserved to enable future analyses that rely on isolate-level information.

Researchers reported *rpoB* numbering using either *Escherichia coli* or *M. tuberculosis* numbering. TBDR uses *M. tuberculosis* numbering and reports the conversion from *E. coli* if and when it is performed. Researchers reported promoter mutations using a variety of genes in the same operon. TBDR normalizes these names only for the *fabG1-hemZ* operon promoter, mutations of which appear in TBDR as the *inhA* promoter. For gyrB, numbering systems have varied [16], and TBDR numbers 714 amino acids (NCBI protein accession number WP_003901763.1).

TBDR was built to provide reproducible results through automated processes. To minimize the manual manipulation of source material, computer programs were written to parse the 1523 data files in the case of the Broad/GTBDR database, an Excel file for TBDReaMDB, and various primary and Supplementary Tables in other publications. The main exception to the automation was PDF document table extraction, which typically required some manual cleanup after a copy and paste.

For published work TBDR stores PubMed identifiers (PMIDs). Since the TBDReaMDB source material did not provide PMIDs, these were identified manually. TBDR connects to PubMed to load reference details via the PMID.

RESULTS

Database Summary
The TBDR database currently contains 39 756 mutations across 29 genes and DST results for 23 drugs and one unspecified fluoroquinolone category. Because some studies performed DST for multiple drugs, the data from 80 studies, including the 73 found in the TBDReaMDB, comprised 148 investigations into drug resistance.

Across the 29 genes, mutations consisted of 1417 distinct amino acid substitutions, 89 distinct regulatory code changes, 105 insertions, and 106 deletions. Table 3 shows the mutations observed in the context of each drug. For example, there were 21 398 mutations observed in isolates subjected to DST for amikacin, and 5556 of these were observed in amikacin-resistant isolates. The numbers in Table 3 are purely descriptive, include mutations measured for genes not expected to be associated with resistance to the particular drug, and do not by themselves inform us about mutation-resistance associations. Table 4 summarizes the number of isolates subjected to DST for each of the drugs in the database. The database content described in Tables 3 and 4 serve as the basis for the calculations and queries described below.

Web Portal to Database
A web portal was established to enable simple access to the database. The portal both facilitated integration efforts and allowed sharing of results among coauthors. There are four main types of tables provided by the portal: (i) a list of drugs with data contained within the database, (ii) a summary of all

Table 2. Online Sources of Data

Description	Compact Citation	Web Site	Comments
TBDReaMDB compiled a comprehensive list of the genetic polymorphisms associated with first- and second-line drug resistance in clinical *M. tuberculosis* isolates throughout the world.	Sandgren et al, 2009 [9]	https://tbdreamdb.ki.se/Info/	High-confidence mutations were integrated into TBDR.org.
Broad's Gates Tuberculosis Drug Resistance Database (Broad/GTBDR). Downloadable isolate mutation and DST results.	None identified	http://www.broadinstitute.org/annotation/genome/mtb_drug_resistance.1/DirectedSequencingHome.html	Download includes 1398 isolates, which were integrated into TBDR.org.

Abbreviations: DST, drug susceptibility testing; TBDR, tuberculosis drug resistance database; TBDReaMDB, tuberculosis drug resistance mutation database.

Table 3. Numbers of Mutations Integrated into TBDR

Drug	Resistant	Susceptible	Total
Amikacin	5556	15 842	21 398
Amoxiclav	240	62	302
Capreomycin	11 407	9415	20 822
Ciprofloxacin	4372	12 170	16 542
Clarithromycin	1233	592	1825
Clofloxacin	17	2418	2435
Cycloserine	129	14 727	14 856
Ethambutol	19 544	8727	28 271
Ethionamide	11 012	6314	17 326
Fluoroquinolones	1091	129	1220
Gatifloxacin	556	518	1074
Isoniazid	28 647	2476	31 123
Kanamycin	5417	11 018	16 435
Levofloxacin	2182	7332	9514
Linezolid	21	1791	1812
Moxifloxacin	1433	3395	4828
Ofloxacin	2650	6525	9175
Para-aminosalicylic Acid	1979	15 247	17 226
Prothionamide	1558	3692	5250
Pyrazinamide	13 370	9094	22 464
Rifabutin	2686	925	3611
Rifampicin	27 150	3842	30 992
Streptomycin	21 250	7497	28 747
Thioacetazone	447	1698	2145

Shown are the numbers of observed mutations at any genetic locus investigated, summed across resistant and susceptible isolates for 23 drugs and the (unspecified) fluoroquinolones category.

Abbreviation: TBDR, tuberculosis drug resistance database.

Table 4. The Number of Isolates Found Resistant or Susceptible to 23 Drugs

Drug	Resistant	Susceptible	Total
Amikacin	593	1622	2215
Amoxiclav	15	4	19
Capreomycin	945	1253	2198
Ciprofloxacin	309	912	1221
Clarithromycin	68	34	102
Clofloxacin	1	137	138
Cycloserine	8	855	863
Ethambutol	2447	2993	5440
Ethionamide	613	372	985
Fluoroquinolones	1048	897	1945
Gatifloxacin	64	58	122
Isoniazid	5142	2299	7441
Kanamycin	655	973	1628
Levofloxacin	148	467	615
Linezolid	1	87	88
Moxifloxacin	170	401	571
Ofloxacin	307	741	1048
Para-aminosalicylic Acid	126	1061	1187
Prothionamide	194	377	571
Pyrazinamide	2350	2503	4853
Rifabutin	259	212	471
Rifampicin	4712	4825	9537
Streptomycin	2594	1486	4080
Thioacetazone	59	331	390

mutations for a given drug, (iii) a list of mutations provided by a reference source, and (iv) a summary for each drug of all canonical mutations for that drug and an array of resistance-mutation statistics. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated on the pooled counts across studies for the following: the number of (i) clinical isolates with both phenotypic and genotypic results for the mutation, (ii) isolates resistant to the drug, (iii) isolates susceptible to the drug, (iv) isolates with the specific mutation, (v) mutant isolates resistant to the drug, and (vi) mutant isolates susceptible to the drug.

Two modes by which the database reports mutations across studies were deemed potentially useful to inform diagnostics research. The first mode merges all mutations with the same amino acid substitution or promoter position. The second mode merges mutations if the nucleotide-level information is also identical. Because some references did not report codon changes and others reported resistance mutations with the nucleotide change but without the codon, the nucleotide-level reports contain multiple rows for identical mutations. The interface allows selecting studies to exclude from the results report.

Results on Drug Resistance-associated SNPs

For each drug category in the TBDR database, mutations were queried to determine which were observed in at least 3 studies, exhibited a nominal specificity greater than 95% and were found at a higher rate in resistant isolates than in susceptible isolates. Many associations identified were noncanonical since resistance mutations for one drug carry information about resistance to another drug tested in the same study. This phenomenon is to be expected, as resistance to multiple drugs is, unfortunately, not uncommon. Table 5 lists the canonical gene-drug associations we used to limit our presentation on drug resistance-associated mutations. Eleven drugs yielded mutations in canonically associated genes that met the above criteria (Table 5). Supplementary Table 1 lists 106 amino acid substitutions and 11 regulatory resistance mutations that were defined by the query. The table is sorted sequentially on three columns: drug, specificity (highest first), and sensitivity (highest first). Table 6 lists the substitutions and regulatory mutations for 2 first-line drugs, isoniazid and rifampicin, sorted as in Supplementary Table 1. This relatively simple query represents a first attempt at using the integrated data. Further refinement by a panel of experts would surely improve the utility of the resistance mutations list for each drug.

There exist mutations in canonically drug-resistance-associated genes that do not reliably predict DST results. Data

Table 5. Drug-gene Associations

Drug	Canonically Associated Genes	Resistance-Associated Genes From TBDR Query (Number of Mutations Matching Criteria)
Aminoglycosides		
Amikacin	*rrs*	*rrs* (2)
Kanamycin	*rrs, eis*	*rrs* (3)
Capreomycin	*rrs, tlyA*	*rrs* (3)
Clarithromycin	*rrl*	NA
Ethambutol	*embB*	*embB* (9)
Ethionamide	*inhA, ethA*	NA
Fluoroquinolones[a]	*gyrA, gyrB*	*gyrA* (7)
Ciprofloxacin	*gyrA, gyrB*	NA
Clofloxacin	*gyrA, gyrB*	NA
Gatifloxacin	*gyrA, gyrB*	NA
Levofloxacin	*gyrA, gyrB*	NA
Moxifloxacin	*gyrA, gyrB*	*gyrA* (5)
Ofloxacin	*gyrA, gyrB*	*gyrA* (7)
Isoniazid	*katG, inhA, ahpC, kasA*	*katG* (5), *inhA* (2)
Linezolid	*rrl, rplC*	NA
Para-aminosalicylic Acid	*thyA*	NA
Prothionamide	*ethA, inhA*	NA
Pyrazinamide	*pncA*	*pncA* (66)
Rifabutin	*rpoB*	NA
Rifampicin	*rpoB*	*rpoB* (16)
Streptomycin	*rpsL, gid, rrs*	*rpsL* (3), *rrs* (8)

Abbreviation: TBDR, tuberculosis drug resistance database.

[a] For studies that reported results for unspecified fluoroquinolones.

integration can help us identify departures from wild type that do not confer drug resistance. This is a specific strength of TBDR, as it brings together the results of multiple studies in a manner that can be queried to address such concerns. For example, TBDR contains *pncA* mutations observed only in pyrazinamide-susceptible isolates and in more than one study, including C14G, S59F, F81S, H82Y, Y103C, and A143T. Similarly, 4 studies found *rrs* 1402 (C->N) mutations in a total of 5 isolates tested for amikacin susceptibility, and all were susceptible, indicating that this mutation may indeed be a poor marker of resistance to this particular drug [17].

Caveats

Table 6 and Supplementary Table 1 identify mutations that are supported by multiple studies and therefore have a higher confidence in their association with drug resistance. These results are not a comprehensive investigation into each drug and mutation, or prediction of resistance to the drug.

The sensitivities for mutations for a given drug in Table 6 and Supplementary Table 1 are not cumulative for predicting drug resistance. First of all, isolates may have multiple mutations and

thus contribute to multiple rows in the table. Second, we do not have all data at the isolate level, as summary tables generally do not preserve this important information. If we had all the data at the isolate level (ie, all mutations reported for each isolate), we could indeed ask what sensitivity (and specificity) combinations of mutations would provide for drug resistance across this idiosyncratic collection of samples.

The predictive value of mutations for drug resistance and diagnostics depends strongly on the proportion of mutation-typed samples that are *a priori* phenotypically resistant. Because the data from many studies are highly biased toward analysis of resistant isolates, the statistics reported are likely quite unreliable as predictions in any particular population. Researchers often avoided typing phenotypically wild-type isolates at their true rates in the population.

DISCUSSION

Integrating tuberculosis drug-resistance data into TBDR required addressing a number of data-handling issues. A straightforward query enabled by the database revealed 96 mutations informative of drug resistance and observed in multiple independent studies.

For sources where complete DST and mutation results were available, TBDR cataloged the association of all resistance mutations with drug sensitivity, including noncanonical associations. Significant noncanonical associations likely arise in part from the evolution of drug resistance to multiple drugs and in part because of ascertainment bias since many studies target populations with MDR and XDR isolates.

The quantitative science required for diagnostics development cannot be addressed fully in an analysis of data such as prepared here. Nevertheless, analyses of existing data should help prioritize mutations. A large impact on diagnostics and patient treatment could be made by using properly integrated data to help the community reach a data-driven consensus regarding tuberculosis drug-resistance predictive mutations.

The Grading of Recommendations Assessment, Development and Evaluation (http://www.gradeworkinggroup.org/) criteria need to be kept in mind when proposing mutations for diagnosis of drug-resistant tuberculosis. A key consideration is how well a mutation actually predicts drug resistance. Because there are associations of particular mutations with phylogeny and of phylogeny with geographic region, these data are important to capture for future drug resistance mutation studies. By including complete or at least expanded sequence results, it should be possible to better understand which mutations are likely to be causal and which are simply markers of resistance in specific populations. It may be important to determine when phylogenetic information has no appreciable impact on mutation-based prediction of drug resistance. The quality of evidence for predicting resistance to drugs will necessarily be better for some mutations than others.

Table 6. Drug Resistance-Associated Mutations for Isoniazid and Rifampicin[a]

Drug	Gene	Mutation	Total Isolates Typed[b]	Resistant Isolates Typed	Susceptible Isolates Typed	Isolates With Mutation	Resistant Isolates With Mutation	Susceptible Isolates With Mutation	Sensitivity	Specificity	PPV	NPV	Number of Studies
Isoniazid	*katG*	S315R	1719	1458	261	46	46	0	3.2	100.0	100.0	15.6	3
Isoniazid	*katG*	del	2424	1172	1252	21	21	0	1.8	100.0	100.0	52.1	6
Isoniazid	*katG*	S315I	4099	2689	1410	18	18	0	0.7	100.0	100.0	34.6	8
Isoniazid	*inhA*	−8 (T/N)	5808	3884	1924	101	100	1	2.6	99.9	99.0	33.7	11
Isoniazid	*katG*	S315N	6227	4239	1988	77	75	2	1.8	99.9	97.4	32.3	15
Isoniazid	*inhA*	−15 (C/N)	6984	4754	2230	895	875	20	18.4	99.1	97.8	36.3	17
Isoniazid	*katG*	S315T	7441	5142	2299	3623	3586	37	69.7	98.4	99.0	59.2	19
Rifampicin	*rpoB*	S450W	8323	3927	4396	68	68	0	1.7	100.0	100.0	53.3	14
Rifampicin	*rpoB*	Q432K	1887	1554	333	11	11	0	0.7	100.0	100.0	17.8	3
Rifampicin	*rpoB*	Q432L	2713	1974	739	11	11	0	0.6	100.0	100.0	27.4	7
Rifampicin	*rpoB*	H445D	9537	4712	4825	155	154	1	3.3	100.0	99.4	51.4	20
Rifampicin	*rpoB*	S441L	6718	2963	3755	24	23	1	0.8	100.0	95.8	56.1	11
Rifampicin	*rpoB*	H445Y	9537	4712	4825	325	323	2	6.9	100.0	99.4	52.4	20
Rifampicin	*rpoB*	H445R	9537	4712	4825	134	132	2	2.8	100.0	98.5	51.3	20
Rifampicin	*rpoB*	D435G	2972	2113	859	33	32	1	1.5	99.9	97.0	29.2	4
Rifampicin	*rpoB*	D435V	9537	4712	4825	293	287	6	6.1	99.9	98.0	52.1	20
Rifampicin	*rpoB*	Q432P	2393	1671	722	8	7	1	0.4	99.9	87.5	30.2	3
Rifampicin	*rpoB*	H445L	9095	4304	4791	96	88	8	2.0	99.8	91.7	53.2	18
Rifampicin	*rpoB*	L452P	9126	4429	4697	141	127	14	2.9	99.7	90.1	52.1	17
Rifampicin	*rpoB*	H445N	7956	3512	4444	40	27	13	0.8	99.7	67.5	56.0	13
Rifampicin	*rpoB*	S450L	9537	4712	4825	2939	2923	16	62.0	99.7	99.5	72.9	20
Rifampicin	*rpoB*	L430P	8375	3881	4494	74	54	20	1.4	99.6	73.0	53.9	14
Rifampicin	*rpoB*	D435Y	5400	3879	1521	73	62	11	1.6	99.3	84.9	28.3	16

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

[a] Detailed results for a total of 11 drugs are included in Supplementary Table 1.

[b] For each row, the quantity in this column is the total number of isolates typed for the mutation irrespective of other mutations.

In short, an effort to include as many isolate-level records (as opposed to summary table information), and to gather enough information to address phylogeny, should allow for analyses that will boost confidence in drug resistance prediction.

Factors other than phylogeny are confusing to evaluate when associating mutations with drug resistance. Importantly, for some mutations, even causal mutations, imperfect correlation with phenotypic DST likely results from varying phenotypic testing methods or drug concentration thresholds across studies. TBDR records information on DST methods reported by different investigators and further analysis could support expert interpretation. Additionally, although some published studies are clearly annotated by geographic region, others are less clearly annotated and also may include isolates from multiple regions.

The integrated data provide a platform for addressing the multivariate properties of the resistance mutations, although we have not demonstrated such an analysis in this article. A multivariate analysis, using statistical, machine-learning, or other mathematical methods, could determine which of the resistance mutations are most informative, and specifically which do not offer additional information when other resistance mutations have been measured. The results of such a study could inform the selection of a panel of resistance mutations that most efficiently uses resources by reducing redundancy.

The current database and web interface are proof-of-principle tools that enabled the generation of the main results as presented herein. The tools demonstrate that data integration is an important component in development of analysis algorithms to identify drug resistance-predictive mutations.

Much of the data we encountered was presented only in summary tables. For data to be most useful to other investigators and inform diagnostics development, information on isolates should always be reported as complete reports on the isolates, including all DST and mutation typing. While most useful would be entry of the data into an appropriate database, at the very least these complete reports should be released as Supplementary Data. For analysis to best demonstrate the diagnostic potential of molecular patterns, it would be most useful to have data from studies that record treatment regimens and outcome data together with mutation and drug susceptibility phenotypes. The completeness of data gathered and other aspects of data quality control should be carefully targeted in future efforts to collect and analyze tuberculosis drug resistance data.

This demonstration of data integration for *M. tuberculosis* drug resistance-associated mutations provides two important lessons. First, the knowledge in the community is currently larger than perhaps has been understood by many researchers and diagnostics developers, and could better inform diagnostic development decisions in the near future. Second, we can anticipate many important issues for gathering and analyzing data with more modern tools, such as bacterial whole genome sequencing, which could better inform microbial profiling efforts in the near future.

A database such as TBDR could be expanded or incorporated into another database to address the growing needs for knowledge sharing with respect to sequence data and markers for tuberculosis drug resistance. To develop a relevant data repository, the database will need to clearly address objectives from the community, namely development of tests for detection of drug resistance and clinical impact. At the same time, to develop a sustainable data repository, appropriate partnerships among researchers, clinical trial groups, reference labs, and commercial entities will need to drive the technology development, as different parties have distinct needs. For example, clinical trial groups are required to anonymize data, and commercial parties may need to compare in-house results with database contents in a confidential manner. As an example, the Critical Path to TB Drug Regimens (CPTR) initiative (http://cptrinitiative.org) has developed strong partnerships with clinical trial groups and commercial entities to tackle the challenges facing tuberculosis drug development. As part of its ongoing work, CPTR has established data management practices and technology that can be adapted to assist needs for knowledge sharing with respect to sequence data and markers for tuberculosis resistance. The power of TBDR and subsequent databases that incorporate existing and prospectively gathered genotypes, phenotypes, and metadata lies in the ability to compose and execute queries. These queries will need to be designed by a collaborative effort among data scientists, stakeholders in diagnostics development, expert committees on tuberculosis drug resistance, and computational biologists. In this way the complexities of different drugs and mutation interactions can be addressed, and a consensus for predicting resistance to each drug should be reached.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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Potential Market for Novel Tuberculosis Diagnostics: Worth the Investment?

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Background. The potential available market (PAM) for new diagnostics for tuberculosis that meet the specifications of the high-priority target product profiles (TPPs) is currently unknown.

Methods. We estimated the PAM in 2020 in 4 high-burden countries (South Africa, Brazil, China, and India) for tests that meet the specifications outlined in the TPPs. The yearly PAM was estimated for the most likely application of each TPP.

Results. In 2020 the PAM for all 4 countries together was estimated to be (1) 12M tests/year with a value of 48M–71M USD for a sputum smear-replacement test; (2) 16M tests/year with a value of 65M–97M USD for a biomarker test; (3) 18M tests/year with a value of 18M–35M USD for a triage test; (4) 12M tests/year with a value of 59M–2238M USD for a tuberculosis detection plus drug susceptibility test (DST) all-in-one or 1.5M tests/year for a DST that follows a positive tuberculosis detection test with a corresponding value of 75M–121M for both tuberculosis detection and DST.

Conclusions. Although there is a considerable potential market for novel tuberculosis diagnostics that fit the specification of the TPPs in the 4 high-burden countries, the actual market for an individual product remains uncertain.

Keywords. tuberculosis; diagnostics; market analysis; market projection; cost; tests; target product profiles.

Recently the World Health Assembly adopted the post-2015 global tuberculosis strategy setting the target for a world free of tuberculosis [1].

Without the introduction of new tools that can cause a more rapid decline in tuberculosis incidence than the current global decline of 2% per year, it is obvious that the post-2015 targets will not be achieved. The development and implementation of new tools and interventions, such as a more effective tuberculosis vaccine, and tuberculosis treatment regimens or more accurate diagnostics that reach more patients are therefore urgently needed.

Recently the tuberculosis community identified the highest needs for new tuberculosis diagnostics [2, 3].

Four of these detailed target product profiles (TPPs) were developed, and the tuberculosis community reached consensus on the most important specifications laid out in each of these TPPs [4, 5]. The 4 TPPs, described in detail elsewhere in this supplement [4] included (1) A point of care sputum-based test as a replacement for smear-microscopy ('smear replacement test'); (2) A point of care, non-sputum-based test capable of detecting all forms of tuberculosis via the identification of characteristic biomarkers or biosignatures, ideally suitable for use at levels below microscopy centers ("non-sputum based biomarker test"); (3) A simple, low cost, point of care triage test, for use by first-contact health care providers as a rule-out test, ideally suitable for use by community health workers ("triage test"); and (4) A rapid drug susceptibility test (DST) that either combines tuberculosis detection and DST into one step ("tuberculosis detection plus DST upfront") or performs tuberculosis detection first and is followed by DST as a second step whenever tuberculosis (or tuberculosis and rifampicin resistance) is detected ("DST after tuberculosis detection test").

Test developers have indicated that apart from clearly specified product requirements, key drivers for them to

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start or continue product development are the time to return on investment, the global market size, the market size on a country level, and the market dynamics [6].

Thus far, several analyses of the tuberculosis diagnostic market have been done, either on a global level or on a country level [7–9]. The tuberculosis diagnostic market has been determined for South Africa and Brazil, and others are underway for China and India [7, 9]. Those assessments focused on the current, served available market of existing tuberculosis diagnostics and did not make any inferences on the potential market of novel tests that target other (new) populations now or in the near future.

In this article, we estimate the potential available market (PAM) for the 4 novel high-priority tests, for which TPPs are in place. This market is described for 4 high-burden countries, being South Africa, Brazil, China, and India, which are part of the BRICS countries (including Russia). The BRICS countries amount to 60% of the total burden of tuberculosis in the 22 high-burden countries and therefore are of special interest for test developers and for tuberculosis control.

METHODS

The potential market in 2020 was estimated both in terms of volume and value for the following 4 selected countries; South Africa, Brazil, China, and India. These countries are emerging economies that are of interest for test manufacturers and have a high tuberculosis burden (they account for 46% of the 6 million tuberculosis cases detected in 2012). The potential market value was calculated by multiplying the projected volume for each of the tests by its lowest and highest price as indicated in the TPP. However, the prices indicated in the TPP are ex-works costs which include the manufacturers' price but do not include any costs related to shipping, import, tax, and distribution. Because there was no consensus reached on the price of the rapid DST TPP, we assumed that the price of the "tuberculosis detection plus DST upfront test" would lie in the range of US$5 to US$20 per test, similar to what was assumed by Pantoja et al [10]. When DST would only follow a positive tuberculosis detection (or rifampicin resistant) test, we assumed that the price of the tuberculosis detection test would be similar to that of a sputum smear-replacement test outlined in TPP 1 (US$5) and that the price of the DST would be between US$10 and US$40 (corresponding with a total of US$15–US$45 for tuberculosis detection and DST).

Using country-specific notification data and prevalence estimates [11], we first determined the potential market per country for each of the TPPs for the year 2012 as a base. For each country, the proportion of tuberculosis patients with pulmonary tuberculosis (PTB), extrapulmonary tuberculosis (EPTB), and children with tuberculosis (assumed to be unable to provide a sputum sample and therefore not included in the number of PTB patients), were estimated separately. Next, the number of prevalent tuberculosis patients in 2012 in each of these categories was

determined, using the World Health Organization's (WHO) estimated prevalence data.

To calculate the number of individuals with signs and symptoms suggestive of tuberculosis that need to be tested to find all prevalent tuberculosis, we applied a country specific "suspect-to-case" ratio, defined as the number of individuals that is being tested in order to find 1 tuberculosis case. For each of the countries this ratio was calculated based on PTB cases and was then extrapolated to other non-PTB cases due to lack of information for the latter. This ratio was either determined based on country specific data on the number of individuals that were screened in 2012 with smear (and/or the Xpert MTB/RIF® assay "Xpert") as the initial test (South Africa and India) or number of smears done for the initials diagnosis (China and Brazil) and the number of notified PTB cases in 2012 dependent on the availability of data.

Because no novel tests that meets the specification outlined in the TPPs is on the market yet, but tests are anticipated to become available within the next 5 years, we estimated the potential market for each of the novel products for the year 2020. The number of prevalent tuberculosis cases in 2020 was estimated based on the 3-year average decline in the tuberculosis prevalence rate and multiplied by the expected population size in 2020 according to the World Bank [12].

For each TPP, the potential market of the base case scenario represented the most likely use of the test with regard to where in the health-care system it would be implemented and its purpose and intended target population (eg, adults and children suspected of PTB, EPTB). In addition, the potential market was determined for alternative scenarios where the test would, for instance:

1. be used on more or less individuals with presumptive tuberculosis than the current estimate by assuming a lower or higher "suspect-to-case" ratio (applicable for all tests but shown for the smear replacement test);

2. be deployed at a lower level of the health-care system and therefore reach a larger population (applicable for the biomarker and triage test); or

3. only be able to test a subset of the intended target population (applicable for the biomarker test or triage test if these would not detect EPTB but only test individuals with presumptive PTB and children with tuberculosis, such as for instance a breath test); or

4. for the DST detection test, would be done after a more sensitive tuberculosis detection test or be done in a staged approach only after rifampicin resistance is found (eg, after Xpert MTB/RIF testing up-front). The different scenarios of each TPP for which we determined the potential market size and value are explained in Table 1. The method we describe and applied for estimating and projecting the potential market size could be used to estimate the potential market in other countries.

Table 1. Base Case and Alternative Scenarios of the Target Product Profiles (TPPs) for Which the Potential Market is Determined

	Scenario	Description
TPP1: smear replacement test	Base case	Sputum-based smear replacement test, deployed at microscopy centers, used for the initial diagnosis in individuals with presumptive PTB
	Plus treatment monitoring	Sputum-based smear replacement test, deployed at microscopy centers, used for the initial diagnosis as well as for treatment monitoring in individuals with PTB. Two additional tests were assumed per diagnosed PTB case for treatment monitoring.
	Low suspect-to case ratio	Sputum-based smear replacement test, deployed at microscopy centers, used for the initial diagnosis in individuals with presumptive PTB. A 'suspect-to-case' ratio of 5 was used to estimate the number of individuals tested to find one PTB case instead of the country specific ratio.
	High suspect-to-case ratio	Sputum-based smear replacement test, deployed at microscopy centers, used for the initial diagnosis in individuals with presumptive PTB. A 'suspect-to-case' ratio of 15 was used to estimate the number of individuals tested to find one PTB case instead of the country specific ratio.
TPP2: biomarker test	Base case	Non-sputum-based biomarker test, deployed at microscopy centers and health-care clinics with a lab attached (equal to a 10% increase compared to deployment at microscopy centers only), used for the initial diagnosis in individuals with presumptive PTB, EPTB or children with tuberculosis.
	Deployment at health posts	Non-sputum-based biomarker test, deployed at health posts (without the necessity of a lab), used for the initial diagnosis in individuals with presumptive PTB, EPTB or children with tuberculosis. An increase of 20% in the number of individuals that get tested was assumed compared to when this test would only be deployed at microscopy centers.
	Deployment at microscopy centers, excluding EPTB testing	Non-sputum-based biomarker test, deployed at microscopy centers and health-care clinics with a lab attached (equal to a 10% increase compared to deployment at microscopy centers only), used for the initial diagnosis in individuals with presumptive PTB or children with tuberculosis.
TPP3: triage test	Base case	Non-sputum-based triage test, deployed at health posts (20% increase in number of individuals tested compared to use at a microscopy centre), used to rule out tuberculosis in individuals with presumptive PTB, EPTB or children with tuberculosis.
	Sputum based test, deployment at health posts	Sputum-based triage test, deployed at health posts (20% increase in number of individuals tested compared to use at a microscopy centre), used to rule out tuberculosis in individuals with presumptive PTB.
	Non-sputum based test, deployment at community	Non-sputum-based triage test, deployed at community care (30% increase in number of individuals tested compared to use at a microscopy centre), used to rule out tuberculosis in individuals with presumptive PTB, EPTB or children with tuberculosis.
TPP 4A: tuberculosis detection plus DST upfront	Scenarios are equal to those described for TPP1. This TPP is not shown separately.	Sputum-based tuberculosis detection and DST in one, deployed at microscopy centers, used for the initial diagnosis of PTB and drug susceptibility testing of at least 1 drug in individuals with presumptive PTB.
TPP4B: DST after tuberculosis detection test	Base case, DST after tuberculosis detection	Sputum-based DST, deployed at microscopy centers, used to test for drug susceptibility in individuals who are diagnosed with PTB. An 80% sensitivity was assumed for the diagnosis of PTB.
	Increased sensitivity of PTB detection (95%)	Sputum-based DST, deployed at microscopy centers, used to test for drug susceptibility in individuals who are diagnosed with PTB. An increased sensitivity of 95% was assumed for the diagnosis of PTB.
	DST detection after detection of RIF resistance	Sputum-based DST, deployed at microscopy centers, used to test for drug susceptibility in individuals who are diagnosed with RIF resistant PTB. A 80% sensitivity was assumed for the diagnosis of PTB. Country-specific prevalence of MDR tuberculosis was used as indicator for RIF resistance prevalence.

Abbreviations: DST, drug susceptibility test; EPTB, extrapulmonary tuberculosis; MDR, multidrug resistant; PTB, pulmonary tuberculosis; RIF, rifampicin.

RESULTS

In 2012 a total of 17 million individuals with presumptive PTB were evaluated for the initial diagnosis of active tuberculosis using the current tests for detection available (smear microscopy or Xpert) in the four countries (Table 2). We estimated that approximately 46% of the individual with presumptive PTB were not tested (range between 12% and 57% for the individual countries). Based on the country specific

Table 2. Key Variables and Assumptions per Country

Variable or Assumption	South Africa		Brazil		China		India		Total		Source
	2012	2020	2012	2020	2012	2020	2012	2020	2012	2020	
Prevalent tuberculosis cases	450 000	426 660	120 000	130 606	1 400 000	962 642	2 800 000	1 628 969	4 770 000	3 148 878	2012: WHO report 2013 2020: estimated based on population size in 2020 according to World Bank and estimated tuberculosis prevalence rate (using country specific 3-year average decline in tuberculosis prevalence rate)
Percentage of all prevalent tuberculosis patients that have PTB	76%	Same as 2012	78%	Same as 2012	98%	Same as 2012	72%	Same as 2012	80%	Same as 2012	WHO report 2013
Percentage of all prevalent tuberculosis patients that have EPTB	14%	Same as 2012	14%	Same as 2012	0.75%	Same as 2012	20%	Same as 2012	14%	Same as 2012	WHO report 2013: % of notified tuberculosis cases with EPTB
Percentage of all prevalent tuberculosis patients that are children with tuberculosis (unable to provide sputum)	10%	Same as 2012	8%	Same as 2012	1%	Same as 2012	8%	Same as 2012	6%	Same as 2012	Calculation: notified smear-positive tuberculosis cases among children assumed to represent 5% of all children with tuberculosis (personal communication A. Mandelakas and B. Kampmann) and 85% of children with tuberculosis assumed to be unable to produce sputum.
Number of individuals with presumptive tuberculosis needed to test to find one tuberculosis case ('suspect to case ratio')	7	Same as 2012	15	Same as 2012	7	Same as 2012	9	Same as 2012	8.3[a]	8.6[a]	Country specific ratio's obtained from NTPs or data from NTPs
Estimated number of individuals with presumptive PTB	2 393 650	2 986 623	1 400 100	1 523 843	9 596 671	6 598 687	18 177 579	10 575 255	31 568 000	21 684 408	Calculation: (number of prevalent PTB cases in 2012 multiplied by the suspect-to-case-ratio)

Table 2   continued.

Variable or Assumption	South Africa		Brazil		China		India		Total		Source
	2012	2020	2012	2020	2012	2020	2012	2020	2012	2020	
Number of individuals with presumptive PTB suspects tested in 2012 (in microscopy centers)	2 116 667 (88% of PTB suspects)	88% of PTB suspects	965 544 (69% of PTB suspects)	69% of PTB suspects	6 173 936 (64% of PTB suspects)	64% of PTB suspects	7 867 194 (43% of PTB suspects)	43% of PTB suspects	17 123 341 (54% of PTB suspects)	54% of PTB suspects	Number of individuals tested with smear and/or Xpert in each country (data provided by NTPs)
Number of individuals with presumptive PTB not tested in 2012	276 983	12% of PTB suspects	434 556	31% of PTB suspects	3 422 735	36% of PTB suspects	10 310 385	57% of PTB suspects	14 444 659	46% of PTB suspects	Calculation: (number of prevalent PTB cases in 2012 multiplied by suspect-to-case-ratio) – (tuberculosis suspects tested in the public and private sector in 2012)
Number of individuals with presumptive EPTB (assumed all not tested in 2012)	441 000	418 127	252 000	274 272	73 500	50 539	5 040 000	2 932 144	5 806 500	3 675 082	Calculation: number of EPTB cases in 2012 multiplied by suspect-to-case ratio
Number of children with presumptive tuberculosis (unable to provide sputum; therefore assumed not tested in 2012)	315 350	298 994	147 900	160 972	129 829	89 271	1 982 421	1 153 322	2 575 500	1 702 559	Calculation: number of children with tuberculosis, unable to provide sputum in 2012 multiplied by suspect-to-case ratio
Total number of individuals with presumptive tuberculosis (PTB, EPTB and children combined) in the country	3 150 000	2 986 623	1 800 000	1 959 087	9 800 000	6 738 496	25 200 000	14 660 721	39 950 000	26 344 927	Calculation: sum of number of individuals with presumptive PTB, EPTB and children with presumptive tuberculosis (unable to provide sputum)

Abbreviations: EPTB, extrapulmonary tuberculosis; NTP, national tuberculosis programmes; PTB, pulmonary tuberculosis; WHO, World Health Organization.
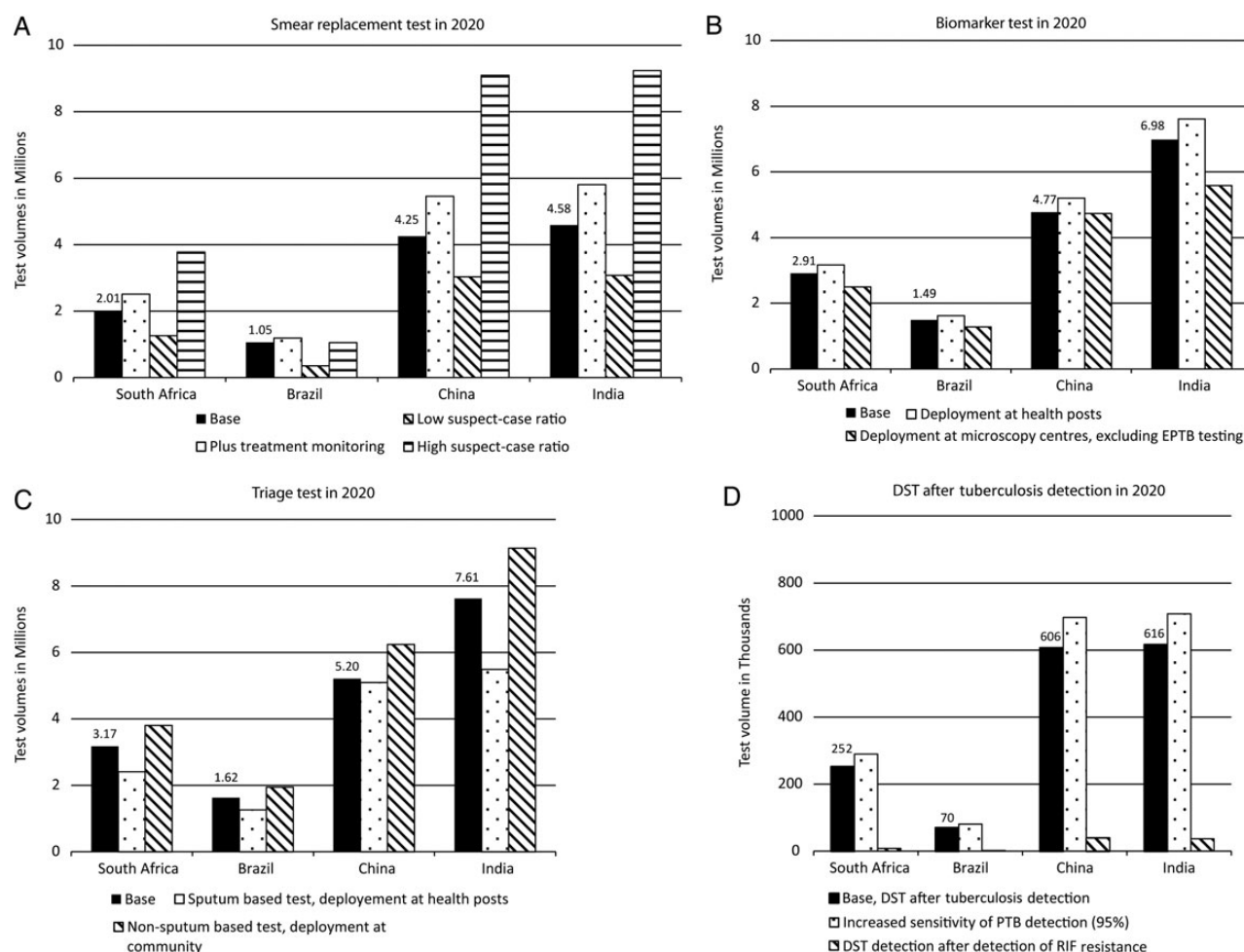
[a] Weighted averages.

Figure 1. Potential available market (volume) for novel tuberculosis diagnostics in 2020 in 4 example countries. *A*, Smear replacement test (TPP 1 and TPP 4A). *B*, Biomarker test (TPP 2). *C*, Triage test (TPP 3). *D*, drug susceptibility test (DST) after tuberculosis detection test (TPP 4B). Abbreviations: PTB, pulmonary tuberculosis; RIF, rifampicin; TPP, target product profile.

"suspect-to-case" ratio, which ranged between 7 in South Africa and China up to 15 in Brazil, we estimated that an additional 5.8 million individuals with presumptive EPTB and another 2.6 million children with presumptive sputum-scarce tuberculosis could have been evaluated in these countries in 2012.

The absolute number of prevalent tuberculosis cases is expected to decline in the coming years in all 4 countries because the population growth rate is smaller than the decline in the tuberculosis incidence rate. The total number of prevalent cases in these 4 countries in 2020 was estimated to be around 3.1 million.

Potential Available Market for a Smear Replacement Test in 2020

For a novel smear replacement test with increased sensitivity for the detection of PTB on sputum that can be deployed at microscopy centers with quick turnaround time, the potential market size in 2020 was estimated at 2.0 million in South Africa, 1.1 million in Brazil, 4.3 million in China, and 4.6 million in

India. This amounts to a total of 12 million tests in that year (Figure 1A). If the smear replacement test could also be used for treatment monitoring and on average 2 additional tests per diagnosed PTB case would be conducted during therapy, the potential market size would grow to 15 million tests per year in all four countries combined. Considering changes in the assumed number of individuals that is tested in order to find one tuberculosis case (eg, lower or higher "suspect-to-case-ratio") the potential market size would vary between 7.7 million (ratio of 5 in all countries) and 23 million tests (ratio of 15 in all countries). The potential market value for a smear replacement test under the base scenario will range between US$48 million for a US$4 test up to US$71 million for a US$6 test in all 4 countries together (Figure 2).

Potential Available Market for a Biomarker Test in 2020

According to its TPP, a novel biomarker test that uses a non-sputum based sample should ideally detect all forms of tuberculosis and be feasible to conduct at least in microscopy centers or
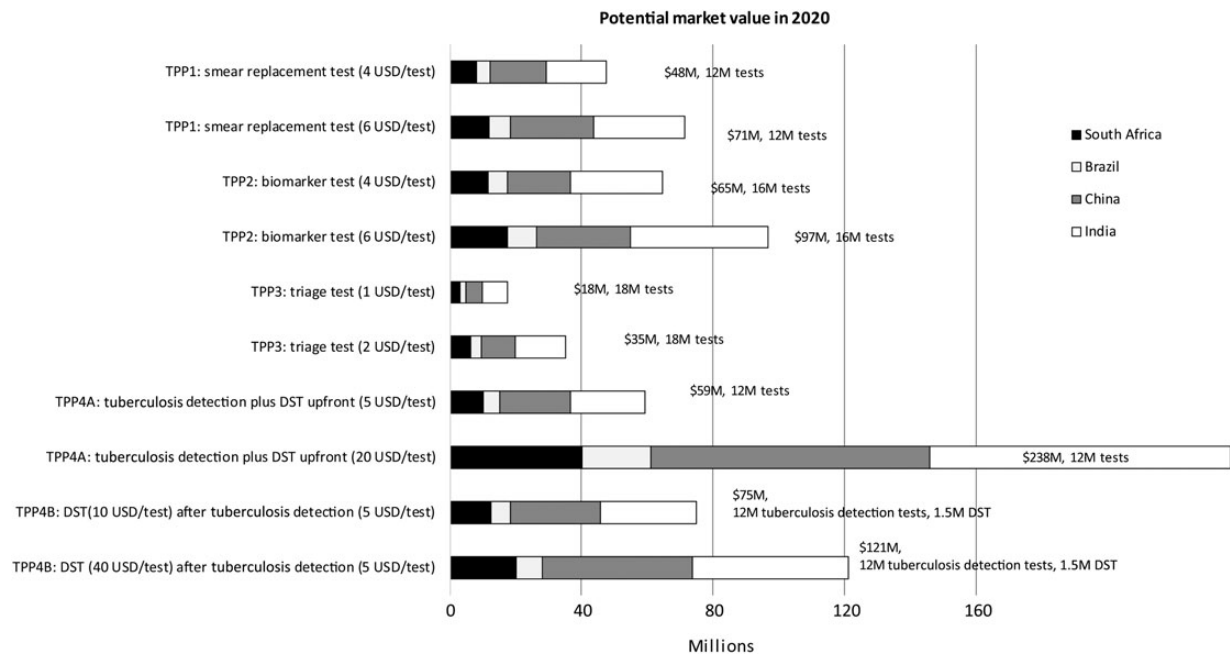
Figure 2. Potential available market (PAM) (value) for novel tuberculosis diagnostics in 2020 in 4 example countries. PAM in 2020 is presented for the base case TPPs at their 2 price points. TPP1 shows the potential market value in 2020 for a TPP for a smear replacement test, deployed at microscopy centers, used for the initial diagnosis of individuals with presumptive pulmonary tuberculosis, and at a price per test of US$4 and US$6. TPP2 shows the potential market value in 2020 for a TPP for a biomarker based test, deployed at microscopy centers and health-care clinics with a lab, used for the initial diagnosis of individuals with presumptive active tuberculosis (all forms), and at a price per test of US$4 and US$6. TPP3 shows the potential market value in 2020 for a TPP for a community triage test, deployed at health posts, used for the screening of individuals with presumptive active tuberculosis (all forms), and at a price per test of US$1 and US$2. TPP4A shows the potential market value in 2020 for a TPP of a tuberculosis detection and drug susceptibility test (DST) in one, deployed at microscopy centers, used for the detection of drug susceptibility in individuals with pulmonary tuberculosis, and at a price per test of US$5 and US$20 for tuberculosis detection and DST combined. TPP4B shows the potential market value in 2020 for a TPP where the DST is conducted after a positive tuberculosis detection. Both tests are deployed at microscopy centers, used for the detection of drug susceptibility in individuals with pulmonary tuberculosis, and at a price of US$5 per tuberculosis detection test and US$10 or US$40 for the DST is assumed. Abbreviation: TPP, target product profile.

health-care clinics with some form of a laboratory attached. Due to its wider applicability, both in terms of target population and in the number of facilities where the test can be conducted, the PAM size in 2020 was estimated at 16.1 million tests for all 4 countries (Figure 1B). Obviously, the market size would increase if the test could be deployed at lower levels of the health-care system such as health posts without a laboratory (total estimated at 17.6 million). On the other hand, if the biomarker test would not be able to diagnose EPTB but would detect only PTB and tuberculosis in children, its market size would be reduced by 13% compared to the base scenario (total market size 14 million tests).

The potential market value for a biomarker test, for the base case scenario, will range between US$65 million for a US$4 test and US$97 million for a US$6 test in total in all 4 countries.

Potential Available Market for a Triage Test

A non-sputum based triage test that is easy to conduct at health posts that do not have a laboratory attached and be used to rule out tuberculosis in individuals with presumptive tuberculosis could have a potential market size of 17.6 million tests in the 4 example countries combined (Figure 1C). In essence, the triage test is expected to have about 10% larger market size than the biomarker test because the test aims to reach difficult to reach populations that did not have access to tuberculosis testing before. Although the potential market size for the TPPs described here is largest for a triage test, its market value is lowest (range between US$18 and US$35 million in total for all 4 countries under the base scenario) because the optimal price range anticipated is US$1 to US$2 per test.

Potential Available Market for a DST in 2020

For a novel (sputum-based) rapid DST there are 2 possible options. First, the test can combine tuberculosis detection and DST into one step (as in the case of Xpert) and test both for the presence of *Mycobacterium tuberculosis* as for resistance against at least one anti-tuberculosis drug in the same sample and in the same test run. For such a test, the potential market

size is equal to that of the smear replacement test (Figure 1*A*), but because this test may cost slightly more, its potential market value in 2020 in all 4 countries is estimated between US$59 and US$238 million for a US$5 to US$20 test (Figure 2).

The second option is that DST only is done after a positive tuberculosis detection test. In this case, the potential market size for the DST would be much smaller with a total of 252 000 tests in South Africa, 70 000 in Brazil, 606 000 in China, and 616 000 in India (Figure 1*D*; a total of 1.5 million tests). Nevertheless, the potential market value for both tuberculosis detection at an average price of US$5 per test followed by DST (at least one drug but preferably more first-line drugs) at a price range between US$10 and US$40 for DST would amount between US$75 million and US$121 million (Figure 2).

DISCUSSION

In this study, we described the PAM in 2020 for 4 novel diagnostics that meet the specification outlined in the TPPs described elsewhere in this supplement [4, 5]. This PAM was determined both in size and in value for 4 countries (South Africa, Brazil, China, and India) that have a high tuberculosis burden but also are emerging economies that can invest in the implementation and rollout of new, modern technologies that have the potential to lead to increased testing and enhanced case detection and which are therefore of interest for test developers.

Product developers need data on issues such as potential global market size, the potential country specific market size, and return on investment, but such information is often lacking (D. Dolinger, FIND, personal communication) [6]. We showed a general approach for estimating the PAM for novel products when used in their intended target population and at their intended level of the health-care system, which can be adapted for other countries or for other assumptions.

Our results indicate that, of the 4 TPPs, the greatest PAM in terms of value would be for a (sputum-based) tuberculosis detection and DST upfront test although this is mainly a result of the high cost per test that we assumed (up to US$20). Such a test, essentially, would be a more sensitive "Xpert"-like test that not only tests for the presence of *M. tuberculosis* and rifampicin resistance but also resistance against additional drugs. Although the potential market looks promising, it is questionable if such a test would be affordable for all countries at this price point [10]. Cost-effectiveness studies on an individual country level are recommended which can take the local epidemiology (eg, prevalence of MDR-tuberculosis) and current testing algorithms in place into account to assess which test strategies are most effective and least costly. Tests that can be deployed at lower levels of the health-care system and which could be used for the detection (or rule-out) of all forms of tuberculosis, such as a biomarker test or a triage test would have the largest potential market volume. And a triage test algorithm might

even be cost-effective even at an even higher price point than what we have used here [13].

In this study, we determined the total PAM for novel tests under the assumption that these would be implemented throughout the whole country and cover 100% of the intended health-care facilities. When different products will reach the market that fit within the same TPP, obviously these products would compete for a share of the same potential available market. Products that meet more of the criteria listed under the "optimal" scenario of the TPPs might account for a larger market share.

In addition, there is interplay between the different TPPs. Although the tuberculosis community has expressed a need for each of the TPPs, and there will be a market for each of them, there is potential overlap in the target populations of some of the tests. Although a triage test and rapid DST are unlikely to compete, a biomarker test for instance will likely replace a smear-replacement test. As a result, there may not only be competition for products that fit the same TPP, but competition could also occur between products that meet different TPPs. The time that novel products will enter the market, the strength of evidence on the test, the recommended use by national and international guidelines of these products in global or local diagnostics algorithms, but also the local epidemiology and preferences will therefore greatly determine the actual market size and penetration.

Several limitations should be taken into consideration when interpreting our results. First, one of the main assumptions in our analysis was the country-specific "suspect-to-case" ratio. Upon changes in this ratio either to a higher or lower number the estimated market size and consequently its volume fluctuated considerably (−35% or +96% when all 4 countries were combined). Although we determined country specific ratios, these were based on the number of individuals with presumptive PTB tested in order to find one PTB case and were assumed to be equal for EPTB and children with tuberculosis (unable to provide sputum), which might not be true. Moreover, we assumed that this ratio would remain constant and not change when tests would be applied at lower levels of the health-care system, while in fact this ratio is likely to increase over time when the prevalence decreases.

In our estimates we used the prevalence estimates according to the WHO. Although these estimates are yearly updated and refined, there is uncertainty around the precise prevalence rates and therefore also the estimates that we presented here for the potential market size for novel test.

Another limitation is that we assumed that an additional 10%, 20%, and even 30% of individuals would get tested when a test would be conducted in health-care clinics with a lab attached, health posts, or in the community besides its use in microscopy centers. Although we did not have accurate data to underpin this assumption, a study conducted by Girosi and Olmsted et al in 2006 estimated that up to 25% of the

population in Africa had access to facilities with no infrastructure, 47% to infrastructure with minimal infrastructure, and 28% to facilities with moderate to advanced infrastructure [14, 15]. Finally, there is uncertainty around the prices of novel tests. The prices used in our calculations should be considered purely indicative as it is hard to predict real prices (which are based on donor investments, special pricing and access agreements, volume-based discounts, etc.).

By 2020, it is highly like that new tuberculosis drug regimens will be available. Because newer drug regimens are critically dependent on companion diagnostics for scale-up, there are ongoing efforts to achieve convergence between diagnostics and new drug regimens [16]. The introduction of newer regimens is not expected to affect the PAM estimates outlined here, unless these will affect current testing practices and for instance lead to an increase in testing during treatment.

In conclusion, we showed that there is a great PAM in the 4 example high-burden countries for novel diagnostics such as a smear replacement test, a biomarker test, a triage test, and DST when these would meet the specifications outlined in the TPPs.

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Costs of Novel Tuberculosis Diagnostics—Will Countries Be Able to Afford It?

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Background. Four priority target product profiles for the development of diagnostic tests for tuberculosis were identified: 1) Rapid sputum-based (RSP), 2) non-sputum Biomarker-based (BMT), 3) triage test followed by confirmatory test (TT), and 4) drug-susceptibility testing (DST).

Methods. We assessed the cost of the new tests in suitable strategies and of the conventional diagnosis of tuberculosis as per World Health Organization guidelines, in 36 high tuberculosis and MDR burden countries. Costs were then compared to the available funding for tuberculosis at country level.

Results. Costs of diagnosing tuberculosis using RSP ranged US$93–187 million/year; if RSP unit cost is of US$2–4 it would be lower/similar cost than conventional strategy with sputum smear microscopy (US$ 119 million/year). Using BMT (with unit cost of US$2–4) would cost US$70–121 million/year and be lower/comparable cost than conventional diagnostics. Using TT with TPP characteristics (unit cost of US$1–2) followed by Xpert would reduce diagnostic costs up to US$36 million/year. Costs of using different novel DST strategies for the diagnosis of drug resistance would be higher compared with conventional diagnosis.

Conclusions. Introducing a TT or a biomarker test with optimal characteristics would be affordable from a cost and affordability perspective at the current available funding for tuberculosis. Additional domestic or donor funding would be needed in most countries to achieve affordability for other new diagnostic tests.

Keywords. costs; tuberculosis; affordability; multidrug-resistant tuberculosis; diagnostics.

Globally, one third of all tuberculosis cases are not identified, in part due to patients not having access to diagnosis or diagnostics being insufficiently sensitive. In addition, low coverage of drug-susceptibility testing (DST) limits the detection of multidrug-resistant tuberculosis (MDR-tuberculosis) [1]. Targets for tuberculosis prevention care and control are to reduce tuberculosis deaths and tuberculosis incidence by 95% and 90% by 2035, respectively, and achieve universal access to drug susceptibility testing [2, 3]. The World Health Assembly recognizes that new diagnostic tools are essential to achieve these new targets [2].

The international community has defined the details of the new diagnostic tests needed in a consultative process led by the World Health Organization (WHO) in

2014 [4–10]. The final report outlining the agreed-upon target product profiles (TPPs) is available [11]. Data on costs, cost-effectiveness, and affordability of new potential diagnostic tests for tuberculosis at a country level are essential information for the international community and test developers alike. There is little available literature assessing the costs and cost-effectiveness of new diagnostics. In fact, the literature is currently restricted to an assessment of the use of a triage test (TT) prior to testing with Xpert® MTB/RIF (Cepheid, Sunnyvale, California) (Xpert). This strategy has been found to reduce total diagnostic costs when compared with using Xpert in all people presumed to have tuberculosis [7].

This article is one in a series of articles in this supplement that discuss the new TPPs for diagnostic tests for tuberculosis and drug-resistant (DR)-tuberculosis, as well as the needs and market potential. In this article, we assessed the total costs and affordability of using new tests as described in the TPPs to diagnose tuberculosis and DR-tuberculosis in 36 high tuberculosis burden and high MDR-tuberculosis burden countries and compared it with the costs of using conventional diagnostics.

METHODS

General Information

We assessed costs from the health system perspective, that is, costs for patients were not taken into consideration, for the year 2012. All values are in 2012 US Dollars. Only costs incurred during the diagnosis of tuberculosis and DR-tuberculosis were considered. Treatment costs were not included in the analysis because the different diagnostic strategies would not change the treatment cost per patient. We acknowledge that the new diagnostic strategies will increase the number of cases identified, and thus the number of cases under treatment will increase, raising total treatment costs. The costs to reach targets set out in the Global Plan have been assessed elsewhere [12].

Setting

Costs were estimated for 36 individual countries that appear in one or both of the lists of 22 high tuberculosis burden countries (22 HBCs) that together account for 81% of the world's tuberculosis incidence, and the 27 MDR-tuberculosis burden countries that account for about 85% of the world's cases of MDR-tuberculosis [1].

Novel Diagnostic Tests

Two tests focused on the detection of tuberculosis either in sputum (a sputum-microscopy replacement test – [RSP]) or on specimens other than sputum (eg, urine, blood, breath; biomarker test [BMT]). A third test aims to make a triage decision (no tuberculosis or very likely tuberculosis; TT). The fourth test focuses on DST, either performing DST [D+DST] in 2 separate steps (ie, 2 reactions), or combining detection and DR-tuberculosis diagnosis [Combined D-DST] in one step (ie, one reaction). Detailed descriptions of these tests can be found in the final meeting report and in the earlier articles in this supplement [11, 13].

Novel Diagnostic Strategies

With the novel diagnostic strategies, each patient requires only one test. A TT is always followed by Xpert as a confirmatory test. For human immunodeficiency virus (HIV)-positive people who are presumed to have tuberculosis but have a negative detection test result with any of the tests (RSP, BMT, or TT), we allow for one confirmatory test using liquid culture. All details of types and quantities of tests required in each diagnostic strategy, and associated sources of evidence, are defined in Table 1.

Conventional Diagnostic

Each new test was compared with the base strategy. The base strategy uses conventional diagnostic algorithms according to the WHO guidelines for all countries. It involves smear microscopy, culture examinations, Xpert, drug susceptibility tests for MDR-tuberculosis on liquid media, and X-rays (Table 1) [14–17]. Xpert is now widely used; therefore, we assume that the number of cartridges sold in 2012 by Cepheid to each country is equal to the number of people screened using Xpert,

allowing for 5% indeterminate results [18]. We assume that Xpert was the first choice for tuberculosis diagnosis among all people with signs and symptoms of tuberculosis present at health facilities. The remaining people presumed to have tuberculosis would be screened and diagnosed using smear microscopy, culture and X-rays. For HIV-positive people who are presumed to have tuberculosis but have a negative Xpert test result, we allow for one confirmatory test using liquid culture [19].

Target Population Considered

All people who present at health facilities with signs and symptoms of tuberculosis are being tested. The size of this population is based on the number of cases notified by each country and the assumption is that there are 10 suspects per 1 smear-positive case notified in 2012 [1]. In line with the TPPs, we assume that the biomarker test reaches 20% more people in all countries, compared to the population reached by the baseline strategy, since it can be performed at lower levels of the health care setting (ie, health posts) [11]. We also assume that the triage strategy reaches 30% more people in all countries because the TT can be performed by a community health worker [11].

Costs Estimation

Costs were estimated using an ingredients approach; this means that costs were calculated by multiplying the unit cost by the quantities of tests required per year [20]. All unit costs and sources of information are shown in Table 2. Unit costs used for the conventional tests (eg, smear microscopy, Xpert)—including capital costs—were based on previous publications [17, 21–24] (Table 2). Unit costs of new tests, and their capital costs, were based on the final report of the TPPs [11] (Table 2). Unit costs for all the tests include only reagents, chemicals, and consumables but exclude costs for labor, overhead, space used, and transport. It is worth noting that all unit costs for the novel diagnostic tests are the result of agreement among the experts consulted for the TPP. In addition, there was no final agreement among experts on the desired optimal unit cost of the test for detection and DST. Therefore, we assumed 3 different unit costs for the DST at US $15, US $30, and US $45 for detection and DST in 2 separate steps (D+DST) and US $5, US $10 and US $20 for detection and DST combined (D-DST). All capital costs (ie, equipment) were annualized using a standard discount rate of 3% [20] and expected years of useful life of 5 years. Additional equipment for smear microscopy, culture, and DST was estimated based on the targets of the Global Plan that aimed at 1 microscopy laboratory per 100 000 people, and 1 culture and/or DST laboratory per 5 million people [12]. This ideal number of laboratories was compared with the current capacity reported by countries [25], and only the cost of equipment was accounted for. All new strategies as well as the conventional strategy use liquid culture as a confirmatory test, therefore all strategies account for investments in equipment for culture as needed per country. The number of G-4 module Xpert

Table 1. Methods—Assumptions for the Strategies Considered

General information
  Analysis per country
  Detailed analysis for 22 HBC and 27 high-MDR-tuberculosis countries (36 countries in total)
  "Suspects" are all people with signs and symptoms of tuberculosis

Strategy	Description	References
Conventional Diagnosis for tuberculosis (Conv)		
Total number of people to be tested	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012	2013 Global TB Report
People to be tested via Xpert	Assume 1 suspect per Xpert cartridge sold in 2012 - data per country (assume 5% waste)	WHO/GLI website
People to be tested via microscopy/culture	Difference between all suspects and suspects to be tested via Xpert	
HIV-positive people to be tested	Number of HIV-positive tuberculosis patients	2013 Global TB Report
Tuberculosis diagnosis		
All people presumed to have tuberculosis	2 smears, 1 x-ray or, 1 Xpert according to the cartridges left after testing tuberculosis in HIV+ people or, 1 culture (liquid) for South Africa, Russia, Estonia and Kazakhstan	WHO guidelines for tuberculosis Country experience
People living with HIV presumed to have tuberculosis	1 Xpert, assume Xpert cartridges first for diagnosing tuberculosis in HIV+ people or, 1 liquid culture if bulk of cartridges not enough	
	1 liquid culture for HIV-positive people in whom Xpert was negative. Assume positivity rate of Xpert among HIV-positive as 79%	Steingart, 2014
MDR-tuberculosis diagnosis		
Numbers of individuals at risk of having MDR-tuberculosis	20% of all new tuberculosis cases + 100% tuberculosis retreatment cases	2013 Global TB Report
MDR-tuberculosis diagnosis	1 culture + DST (liquid media)	WHO guidelines for MDR-tuberculosis
Number of additional laboratories needed	Assume 1 microscopy laboratory per 100 000 population (ideal)	Global Plan
	Assume 1 culture laboratory per 5 million population (ideal)	Global Plan
	Assume 1 DST laboratory per 5 million population (ideal)	Global Plan
	Existing number of microscopy and culture laboratories	2013 Global TB Report
	Additional laboratories needed are the difference between ideal number and existing number	
	Each additional laboratory equipped for microscopy or culture or DST	
Number of G-4 Xpert machines to buy	Number of Xpert cartridges sold in 2012 divided by 3000	WHO/GLI website
	Assume each machine does 3000 tests per year	WHO – expert opinion
Rapid Sputum-based Test (RSP)		
Total number of people to be tested	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012. Sensitivity of 95% (optimal sensitivity for *M. Tuberculosis* detection)	2013 Global TB Report TPP final report
Diagnosis of tuberculosis	1 RSP test per suspect, 1 x-ray for those with negative result	TPP final report
	1 liquid culture for HIV+ people suspected of having tuberculosis and negative result on RSP. Sensitivity of 80% of RSP among HIV-positive	TPP final report
Number of instruments needed	1 instrument per microscopy laboratory	TPP final report
	Assume 1 microscopy laboratory per 100 000 population	Global Plan
Number of additional laboratories needed	For liquid culture, assume 1 culture laboratory per 5 million population (ideal)	Global Plan
Biomarker Test (BMT)		
Total number of people to be tested	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012. Additionally, 20% more than conventional assumption due to possible implementation at lower levels of the health care system and diagnosis of extra-pulmonary tuberculosis and sputum– scarce tuberculosis with non-sputum sample	2013 Global TB Report TPP final report
Diagnosis of tuberculosis	1 test per suspect. Sensitivity of 98% (optimal sensitivity) and prevalence rate of 10%. Confirmatory testing only for HIV-positive people.	TPP final report
	1 liquid culture for HIV+ people in whom the BMT was negative. Sensitivity of BMT among HIV-positive as 80%.	TPP final report
Number of additional laboratories needed	For liquid culture, assume 1 culture laboratory per 5 million population (ideal)	Global Plan

Table 1 continued.

General information		
Analysis per country		
Detailed analysis for 22 HBC and 27 high-MDR-tuberculosis countries (36 countries in total)		
"Suspects" are all people with signs and symptoms of tuberculosis		

Strategy	Description	References
Triage Test (TT)		
Total number of people to be tested	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012. Additionally, 30% more than conventional assumption for all countries due to possible implementation at lower levels of the health care system and diagnosis of extra-pulmonary tuberculosis and sputum- scarce tuberculosis with non-sputum sample	2013 Global TB Report TPP final report
Number of suspects to be tested with confirmatory test, ie, with positive result of triage test	Assumed as 95% (optimal sensitivity for *M. Tuberculosis* detection independent of smear-status as per TPP), 80% specificity and 10% prevalence rate.	TPP final report
Diagnosis of tuberculosis	1 test per suspect	TPP final report
	1 confirmatory test if TT is positive using Xpert	TPP final report
	1 liquid culture for HIV-positive people in whom the TT was negative. Assume sensitivity of TT among HIV-positive as 95%.	TPP final report
Number of G-4 Xpert machines to buy	Number of Xpert tests needed divided by 3000	WHO – expert opinion
Number of additional laboratories needed	For liquid culture, assume 1 culture laboratory per 5 million population (ideal)	Global Plan
Next generation of DST, detection plus a novel DST, two step testing – strategy described in TPP under minimal scenario (New D+DST)		
Total number of people to be tested for tuberculosis	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012	2013 Global TB Report
Total number of people to be tested with the novel DST	All cases that were identified positive from the detection test: assumed as 95% (which is the optimal sensitivity of the proposed RSP)	TPP final report
Diagnosis of tuberculosis	1 test per suspect Assume rapid sputum-based test, with sensitivity of 95% and optimal unit cost (optimal scenario for *M. Tuberculosis* detection)	TPP final report
Diagnosis of DR-tuberculosis	1 test per tuberculosis case	TPP final report
Number of new instruments needed	1 new instrument per microscopy laboratory	TPP final report
	Assume 1 microscopy laboratory per 100 000 population	Global Plan
Combined detection and DST - Next generation of DST - strategy described in TPP under minimal scenario (New combined D-DST)		
Number of people to be tested for tuberculosis - AND - number of people to be tested with the novel DST combined	Assume 10 suspects per 1 smear-positive tuberculosis case notified in 2012	2013 Global TB Report
Diagnosis of tuberculosis and DR-tuberculosis	1 test per suspect	TPP final report
Number of new instruments needed	1 instrument per microscopy laboratory	TPP final report
	Assume 1 microscopy laboratory per 100 000 population	Global Plan

Abbreviations: BMT, biomarker test; D+DST, detection and drug-susceptibility testing in two separate steps; D-DST, detection and drug-susceptibility testing in one step; DR-tuberculosis, drug-resistant tuberculosis; DST, drug-susceptibility testing; HBC, high tuberculosis burden country; HIV, human immunodeficiency virus; *M. Tuberculosis, Mycobacterium Tuberclosis*; MDR-tuberculosis, multidrug-resistant tuberculosis; RSP, rapid sputum-based test; TPP, target product profile; TT, triage test followed by Xpert; WHO, World Health Organization.

machines were estimated based on the assumption that one machine handles about 3000 tests per year [17].

Results are available for each of the 36 individual countries upon request. In this article we show results for all 36 countries as a group. Supplement material shows results for the groups of 22 high-burden countries, of 27 high MDR-tuberculosis burden countries, and of BRICS countries.

Affordability Analysis

Affordability was assessed by comparing the costs of the new and conventional strategies with the funds that countries are currently spending on tuberculosis. In particular, the costs of conventional diagnostics relative to available funding for tuberculosis control were compared with costs of new diagnostics relative to available funding for tuberculosis [25]. Countries report the budget for their National TB Programme on an annual basis to the Global TB Programme at WHO [1]. Out of the 36 countries, 2 countries did not report financial data; therefore, results for each of the 34 individual countries are available upon request. In this article we show results for the 34 countries as a group. Methods used for this assessment have been described in further detail prior to this study [22].

Analyses were performed using STATA/SE 13.1.

Table 2. Methods—Unit Costs, US$, 2012 Prices

	Unit Cost ($)	Quantities	Reference
Diagnostic Tests for tuberculosis			
Smear[a]	0.61	2	WHO P&B Tool, tab "LabItemsList", line 526
X-ray	1.5	1	Recent experience in tuberculosis prevalence surveys
Culture (liquid media)[a]	5.66	1	WHO P&B Tool, tab "LabItemsList", line 532 and 533
Xpert	9.98	1	WHO P&B Tool, tab "LabItemsList", line 551
BMT[a] (Low, Medium, High)	2, 4[b], and 6[c]	1	TPP final report
TT[a] (Low, Medium, High)	1[b], 2[c], and 4	1	TPP final report
RSP[a] (Low, Medium, High)	2, 4[b], and 6[c]	1	TPP final report
Diagnostic Tests for MDR-tuberculosis and DR-tuberculosis			
DST (4 drugs – liquid media)[a]	15		WHO P&B Tool, tab "LabItemsList", line 539 and 540
New D+DST: tuberculosis detection[a]	4	1	TPP final report
New D+DST: DST[a] (Low, Medium, High)	15, 30 and 45	1	Assumption
New combined D-DST, (Low, Medium, High)	5, 10 and 20	1	TPP final report
Laboratory Equipment[d]			
AFB laboratory, per new laboratory	19 624	1	WHO P&B Tool
Culture in solid media, per new laboratory	198 098	1	WHO P&B Tool
(Culture and) DST in solid media, per new laboratory	204 005	1	WHO P&B Tool
MGIT for liquid culture and DST, per new laboratory	79 655	1	WHO P&B Tool
MGIT for liquid culture and DST for countries for which FIND has negotiated prices, per new laboratory	38 950	1	WHO P&B Tool and FIND
GeneXpert machine, 4 modules	17 500	1	Xpert *M. Tuberculosis*/RIF implementation manual
Shipment, Printer, UPS	2400	1	Xpert *M. Tuberculosis*/RIF implementation manual
Annual calibration Xpert (First year)	450	1	Xpert *M. Tuberculosis*/RIF implementation manual
New instrument for RSP (Low/Medium and High)	500 and 1400	1	TPP final report
New instrument for novel DST	1900[e]	1	TPP final report
New instrument for combined D-DST	1400	1	TPP final report

Abbreviations: BMT, biomarker-test; D+DST, detection and drug-susceptibility testing in two separate steps; D-DST, detection and drug-susceptibility testing in one step; DR-tuberculosis, drug-resistant tuberculosis; DST, drug-susceptibility testing; *M. Tuberculosis*, *Mycobacterium Tuberculosis*; MDR-tuberculosis, multidrug-resistant tuberculosis; RSP, rapid sputum-based test; TPP, target product profile; TT, triage test followed by Xpert; WHO, World Health Organization.

[a] Unit cost for these tests includes reagents, chemicals, and consumables. It excludes costs for labor, overheads, space use, and transport.

[b] Unit cost expected in the optimal scenario.

[c] Unit cost expected in the minimal scenario.

[d] Costs for infrastructure, annual maintenance and quality assurance are not included.

[e] Includes cost of the equipment for RSP (US $500) and the equipment cost for the novel DST (US $1400).

RESULTS

Numbers of Tests and Costs

An estimated 21 million people presumed to have tuberculosis in the 36 high tuberculosis and MDR-tuberculosis-burden countries were tested using the conventional methods, with an estimated total cost of US $119 million in year 2012 (Figure 1, Table 3). Out of the 21 million people tested, 1.1 million people were tested using Xpert. Costs of using Xpert accounted for 13% of total costs of using the conventional methods. Capital investments in smear microscopy laboratories and the recurrent costs of smears accounted for the largest share of total costs using conventional diagnostics, 25% and 20%, respectively. The cost per year of using a rapid sputum-based test for tuberculosis detection [RSP] for all suspects currently tested with smear (21 million people) was US $93–187 million (lower value calculated

using the lower unit cost of US $2 per test and upper value calculated using the higher unit cost of US $6). The total cost of using a biomarker tuberculosis detection test [BMT] ranged from US $70 to US $172 million (lower value using lower unit cost of US $2 and upper value with higher unit cost per test of US $6) to test 26 million people (20% increase in number of patients reached). Using a community-based TT for 28 million people (30% increase in number of patients reached) presumed to have tuberculosis would result in estimated total costs between US $83 million (unit cost per test US $1) and US $165 million (high unit cost per test of US $4). This TT scenario includes using Xpert as a confirmatory test for an estimated 3.2 million people who test positive on TT.

Overall, the use of the 3 new diagnostics (RSP, BMT, and TT) would reduce diagnostic costs compared to the cost of conventional methods if the unit cost per test is US $2 or less (Figure 1).
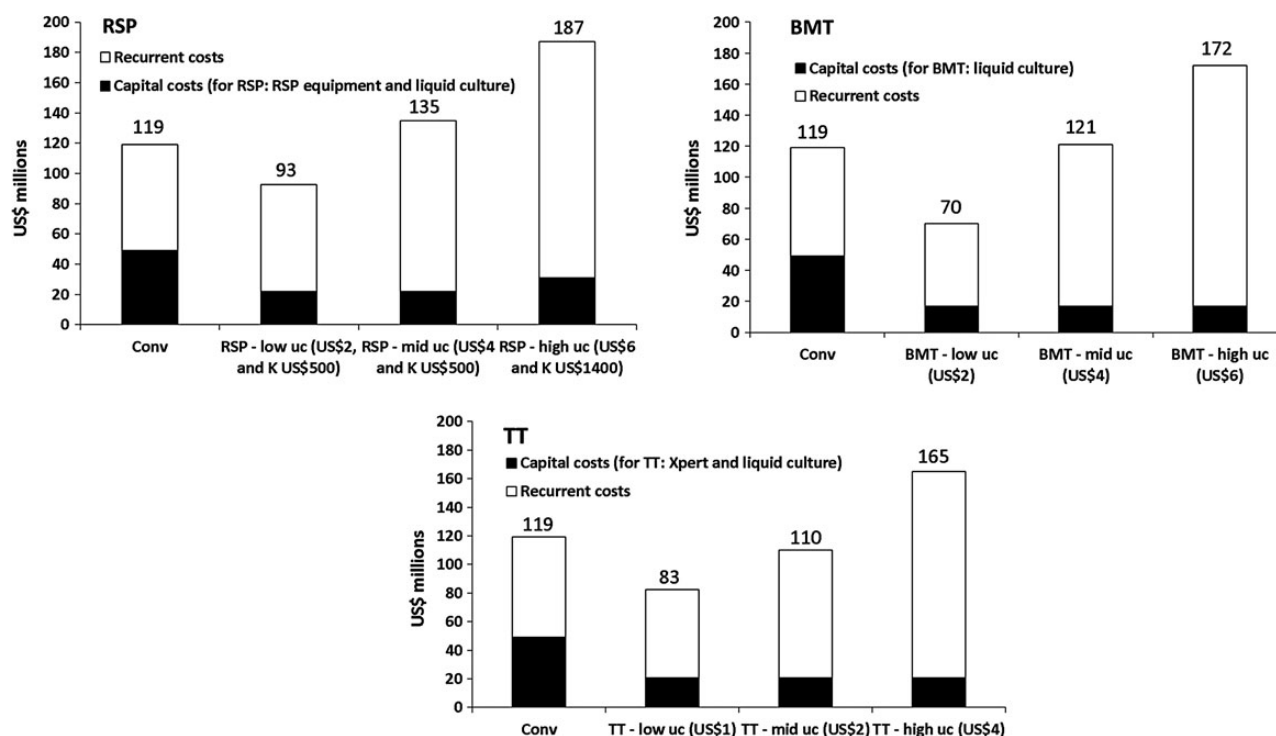
Figure 1. Estimated costs of diagnosing tuberculosis using conventional methods (Conv) compared with the costs of using a rapid sputum-based test (RSP), biomarker test (BMT), and triage test followed by Xpert (TT), US$, year 2012, 36 focused countries. Capital costs (K) include only equipment. Recurrent costs (uc) include reagents, chemicals and consumables of the test. Capital costs for the conventional diagnostics include investments for equipment for smear laboratories, for laboratories for culture in liquid media and Xpert machines.

The cost of using the rapid sputum-based test with a unit cost of US $4 (together with equipment cost of US $500) resulted in an increase in diagnostic costs by 13% compared to the cost of using conventional methods. Diagnostic costs increased by 57% in the 36 countries when using the rapid sputum-based test with a higher unit cost per test of US $6 and equipment costs of US $1400. Using a biomarker test with a unit cost of US $4 resulted in similar diagnostic costs compared with the cost of using of conventional methods. However, using a biomarker test with a high unit cost per test of US $6 increased the diagnostic cost by 44% in the 36 countries compared to the costs of using conventional diagnostics. The triage strategy—TT followed by Xpert—reduced diagnostic costs by 31% at a unit cost per test of US $1, and by 7% at a unit cost of US $2 in the 36 countries compared with the costs of using conventional methods. At a higher unit cost of US $4, the use of a triage strategy increased diagnostic costs by 38% compared to the cost of using conventional methods.

Results as described above show a common pattern among the individual 36 countries, with the exception of Russia and South Africa. In these 2 countries, the cost of using any of the new strategies with any of the three unit costs per test seemed to be less than using conventional diagnostics; the reason being that costly culture is routinely integrated into the algorithm for the diagnosis of tuberculosis. Results for the 22 high-burden countries as a group, for the 27 high MDR-tuberculosis burden countries as a group and for the group of Brazil-Russia-India-China-South-Africa (BRICS) are available in Supplement material. Results for each country are available upon request.

The total cost of diagnosing tuberculosis and MDR-tuberculosis using conventional methods is estimated at US $162 million for the year 2012 (Figure 2, Table 3). This included the cost of using liquid culture and DST for diagnosis of MDR-tuberculosis in an estimated 1.5 million tuberculosis cases. Total costs of using a new detection test for all people with signs and symptoms of tuberculosis followed by a novel DST for all tuberculosis cases [New D+DST] ranged from US $179 to US $240 million (lower value corresponds to lower unit cost per DST test of US $15 and upper value to higher unit cost per DST test of US $45). Using a combined test for diagnosis of tuberculosis and detection of drug resistance up-front [New combined D-DST] resulted in estimated total costs between US $165 million and US $484 million (lower value for a low unit cost per test of US $5; higher value using a high unit cost per test of US $20).

Affordability at a Country Level
The affordability of each alternative strategy in 34 countries as a group is illustrated in Figures 3 and 4 (Supplementary Tables 1

Table 3. Estimated Number of Tests and Estimated Total Costs by Strategy, 36 Focused Countries, Year 2012

Diagnostic Test	Estimated Number of Tests—People Presumed to Have tuberculosis (Millions)	Estimated Costs (US$ Millions)	Unit Cost Per Person Tested (US$)
Conventional method for tuberculosis	21	119	5.6
Initial diagnosis using Xpert[a]	1.1		
RSP	21		
Low unit cost US $2		93	4.4
Medium unit cost US $4		135	6.4
High unit cost US $6		187	8.9
BMT[b]	26		
Low unit cost US $2		70	2.8
Medium unit cost US $4		121	4.7
High unit cost US $6		172	6.7
TT[c]	28		
Confirmatory test using Xpert	3.2		
Low unit cost US $1		83	3.0
Medium unit cost US $2		110	4.0
High unit cost US $4		165	6.0
Conventional method for tuberculosis and MDR-tuberculosis	21	162	7.7
Tuberculosis cases tested for MDR-tuberculosis	1.5		
New D+DST	21		
Tuberculosis cases tested for MDR-tuberculosis	2.0		
Low unit cost US $15		179	8.4
Medium unit cost US $30		210	9.9
High unit cost US $45		240	11
New combined D-DST	21		
Low unit cost US $5		165	7.8
Medium unit cost US $10		271	13
High unit cost US $ 20		484	23

Abbreviations: BMT, biomarker test; D+DST, detection and drug-susceptibility testing in two separate steps; D-DST, detection and drug-susceptibility testing in one combined step; MDR-tuberculosis, multidrug-resistant tuberculosis; RSP, rapid sputum-based test; TT, triage test followed by Xpert.

[a] Number of tests of Xpert were based on sales volumes in 2012, please refer to the Methods for more details.

[b] Number of tests of the biomarker assumes 20% additional people to test.

[c] Number of tests of the triage strategy assumes 30% additional people to test.

and 2 illustrates results for each country). In the 34 countries, the cost of using conventional methods with the goal of reaching the Global Plan targets for tuberculosis diagnosis represented around 11% of the currently available funding for tuberculosis, with a maximum of 96% (Democratic Republic of the Congo) and a minimum of 0.2% (Russia). The cost of using a RSP was between 8% and 16% of the available funding for tuberculosis in the 34 countries. The cost of using the BMT as a proportion of the available funding for tuberculosis was between 6% and 16% for the 34 countries. Similarly, the cost of using the triage strategy—followed by Xpert—ranged between 8% and 15% of the available funding for tuberculosis for the 34 counties as a group.

For the diagnosis of tuberculosis and MDR-tuberculosis, the cost of using the conventional methods was around 14% of the available funding for tuberculosis (Figure 4). For the 12 low-income countries, it represented 16% of the available funding

for tuberculosis (more details in Supplementary Tables 1 and 2). Costs of diagnosing tuberculosis and DR-tuberculosis using a 2-step novel diagnostic test represented between 16% and 21% of the available funding for tuberculosis in the 34 countries considered. Using one single test to diagnose tuberculosis and DR-tuberculosis would take up the highest proportion of available funding compared to the other strategies, ranging between 15% and 44% for the 34 countries.

DISCUSSION

This is the first study to our knowledge to assess the costs and affordability of the new diagnostics as described in the TPPs for detection of tuberculosis and DR-tuberculosis in 36 high tuberculosis and high MDR-tuberculosis burden countries. This study provides further information on the boundaries of the unit costs that would be affordable for countries.
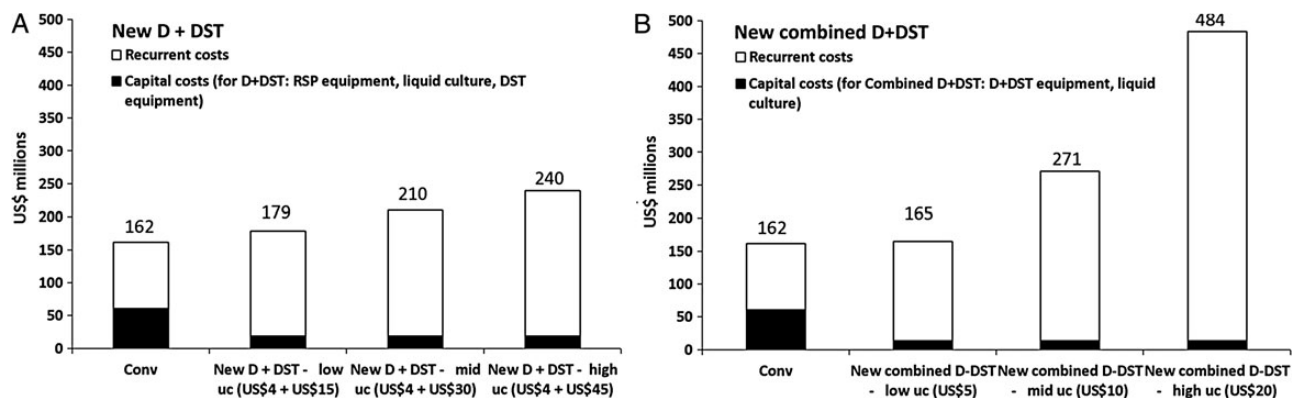
Figure 2. Estimated costs of diagnosing tuberculosis and drug-resistant tuberculosis using conventional methods (Conv) compared with costs of using: A) detection and DST in two separate steps (New D+DST), and B) detection and DST in one step (New combined D-DST), US$, year 2012, 36 focused countries. Capital costs (K) include only equipment, recurrent costs (uc) include reagents, chemicals and consumables of the test. Capital costs for the conventional diagnostics include investments for equipment for smear laboratories, for laboratories for culture and DST in liquid media and Xpert machines. Abbreviations: DST, drug-susceptibility testing; RSP, rapid sputum-based test.

Our results suggest that a triage strategy, with both minimal and optimal characteristics [11], followed by a confirmatory test like Xpert, reduces the costs of diagnosing tuberculosis in all 36 countries compared to the use of conventional diagnostic methods, as well as compared to the use of a rapid sputum-based test. This analysis also supports a recent hypothetical cost-effectiveness analysis that suggested that a TT prior to Xpert implemented at the same level as Xpert (with a TT
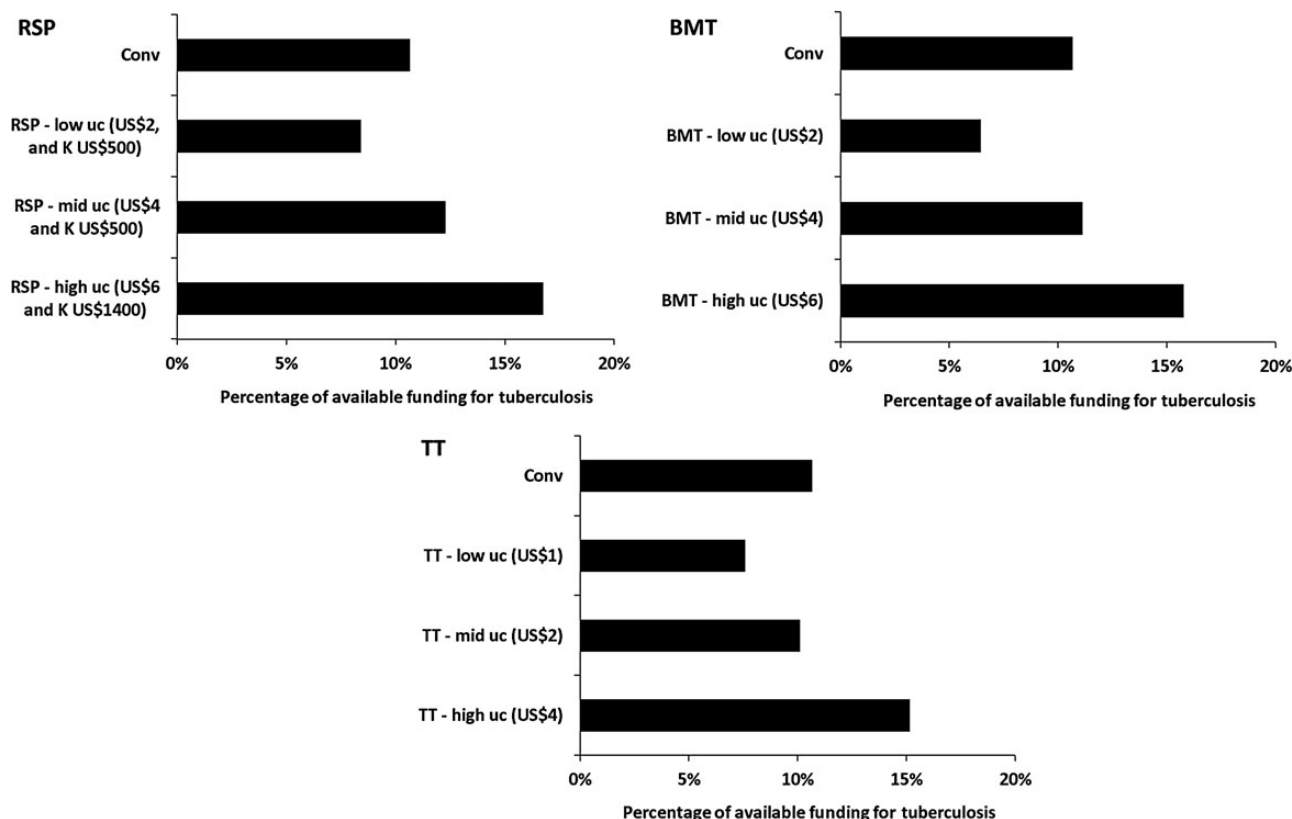
Figure 3. Annual costs of diagnosing tuberculosis using conventional methods (Conv), rapid sputum-based test (RSP), biomarker test (BMT) and triage test followed by Xpert (TT), as a proportion of countries available funding for tuberculosis, %, year 2012, 34 countries. Financial data not available for Azerbaijan and Lithuania.
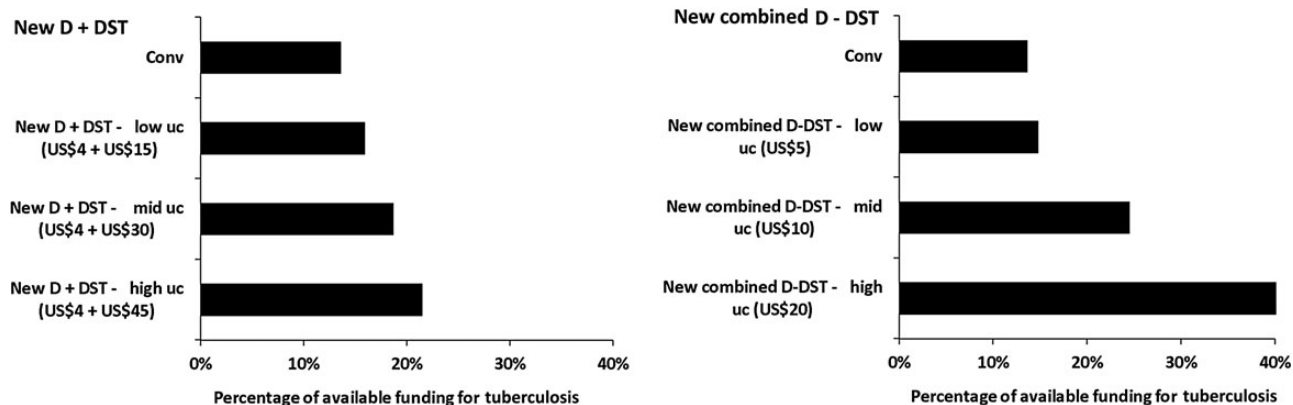
Figure 4. Annual costs of diagnosing tuberculosis and drug-resistant tuberculosis using conventional methods (Conv), detection and drug-susceptibility testing (DST) in two separate steps (New D+DST), and detection and DST in one step (New combined D-DST), as a proportion of countries available funding for tuberculosis, %, year 2012, 34 countries. Financial data not available for Azerbaijan and Lithuania.

with a sensitivity equal to Xpert and a specificity of 75%) would reduce diagnostic costs even at a high unit cost of US $5 in the 3 countries sampled (Uganda, India, and South Africa) [7]. Our analysis further considers implementation of the triage strategy at the community level, which would improve coverage (it is assumed that it would reach 30% more people), compared to the use of conventional diagnostics, and still the TT would remain affordable (although increased treatment costs were not factored in).

A rapid sputum-based test with a better sensitivity compared to microscopy would increase the number of patients diagnosed and at a unit cost per test up to US $4 would result in similar or lower costs compared to the cost of using conventional diagnostics. Even the biomarker test with an expected increase in coverage of 20% would reduce diagnostic costs under optimal characteristics with a unit cost per test below US $4.

The next generation of DSTs is intended to be used at lower levels of the health-care system and has better sensitivity compared to current methods. At the costs anticipated in this article, these tests would result in similar or higher diagnostic costs compared to the cost of using conventional methods. A test that first detects tuberculosis and then identifies drug resistance in a second step at US $15 would approach the cost of the conventional strategy. Only a test that performs detection and DST in one step [Combined D-DST] with a unit cost at or below US $5 would be cheaper than conventional diagnostics. However, currently no diagnostic solution is likely to meet such a price point when detection and DST are combined.

Although our calculations are conservative, there are several factors that limit our analysis. First, the number of people with signs and symptoms of tuberculosis to be tested is based on the assumption that there are 10 suspects per 1 smear-positive tuberculosis case notified in 2012. A recent publication that collected country data on number of sputum smears tests done in

2012 [26] shows that our baseline number of suspects may be an underestimate. However, any change in size of the population requiring tests will affect all strategies largely in the same way and therefore does not affect this relative comparison of costs. Second, we did not consider the cost implications of the alternative strategies for patients and their families. In theory, the TT and BMT strategy should reduce costs to patients by facilitating access to diagnosis at the community level, but is not reflected in our current calculations. Third, labor and transport (mainly for culture samples) were not included in the unit cost of any tests. Therefore, it is possible that costs have been underestimated. Fourth, we used one single unit cost for each test in the conventional diagnostics from the WHO budgeting tool, because this tool is used at country level for budgeting. However, we acknowledge that there is great variety in the unit costs of culture and DST tests among published articles. Fifth, novel tests for DST may include a broader portfolio of drug resistance tests. Conventional diagnosis of DR-tuberculosis, however, only detects resistance against first line drugs. This potential added benefit of novel tests is not reflected in our current costs. Sixth, capital costs in the conventional strategy are based on the assumptions of the Global Plan in terms of number of laboratories required per population. We recognize that the capital investment in some countries could be higher than is anticipated by the Global Plan.

We have defined affordability by comparing the costs of diagnosis to the current levels of available funding at country level. However, current available funding entails great variability across countries. Recent analyses show that BRICS and upper-middle income countries are increasingly able to mobilize resources for almost all their funding needs from domestic sources [27, 28]. In contrast, low-income countries rely mostly on donor funding to meet their financial needs. Donor funding accounted for 67% of total funding in low-income countries

in 2011. The estimated funding gap to reach targets set in the Global Plan to Stop TB is substantial and requires much more resources to be mobilized domestically and from donors.

Costs faced by patients and their families during the diagnostic pathway for tuberculosis can represent on average up to 53% of annual household income per capita [29]. Novel diagnostics for tuberculosis that could reduce the financial burden faced by families are needed. The novel diagnostic tests modeled herein theoretically will reduce the financial burden for patients either through use closer to the patient or improved accuracy.

We greatly encourage further cost-effectiveness and transmission modeling to evaluate the implications of the new tests and to determine the most cost-effective algorithm using detailed country data.

CONCLUSIONS

New methods for diagnosing tuberculosis and DR-tuberculosis are essential to improve tuberculosis prevention, care, and control. Our results suggest that from a cost and affordability perspective, introduction of a TT (followed by Xpert) or a biomarker test (with optimal characteristics as defined in the TPP) would reduce diagnostic costs and improve coverage compared to the conventional diagnosis that relies on smear microscopy. To ensure affordability of the RSP and of the next generation of DSTs, further funding for tuberculosis at the country level is needed or the lowest unit cost for the new tests must be achieved.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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Discovery, Innovation, and New Frontiers in Tuberculosis Diagnostics: Reflections and Expectations

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On the evening of 24 March 1882, Robert Koch (1843–1910) announced to the Berlin Physiological Society that he had discovered the cause of tuberculosis. He had conclusively stained bacilli in lung tubercles from animals infected with tuberculosis, a discovery that proved to be a turning point for the scientific world in understanding the deadly disease that had plagued humankind for millennia.

In the audience that evening was a young Paul Ehrlich (1854–1915). A centennial paper commemorating Koch's discovery of the tubercle bacillus [1] describes some rapid innovations following Koch's announcement: Ehrlich (who recalled having seen, in various materials including sputum, bacilli similar to those demonstrated by Koch), obtaining from Koch a pure culture of tubercle bacilli immediately after the lecture, and on the same evening starting to experiment with various stains that he (Ehrlich) had already devised [1]. His first innovation was a shorter staining time and applying acid and alcohol for a few seconds to decolorize the surrounding tissues while the tubercle bacilli retained the primary stain and became more clear [1].

The next innovation happened overnight, by accident [1]. Apparently, Ehrlich left the stained preparations to dry on top of a cold stove in his laboratory. The next morning he was annoyed to find that the stove had been lit, but when he examined the slides he was astonished to find the bacilli in clumps showing up even more clearly [1]. The benefit of heating slides had just been shown. More innovations followed rapidly; Ziehl introduced carbolic fuchsin instead of aniline as a dye, whereas Neelsen advocated the use of sulphuric instead of nitric acid, and the famous "Ziehl–Neelsen" staining technique and the "acid-alcohol fast bacillus" were born [1].

Subsequent progress in tuberculosis diagnosis and drug susceptibility testing (DST) was, however, painstakingly slow. Culture of the tubercle bacilli proved to be difficult. Koch initially used solid culture medium developed from inspissated cattle-blood serum. Several innovations by other microbiologists followed, until eventually an enriched egg-based solid medium developed by Löwenstein and Jensen in 1932 became the first "gold standard" for culture and DST. The idea of using liquid synthetic media was first introduced in 1892 [2]; however, progress was plagued by the slow growth of *Mycobacterium tuberculosis*, culture overgrowth by other micro-organisms, and the biohazards of manipulating suspensions containing a high number of tubercle bacilli. Innovation stagnated, and a new "gold standard" for culture and DST only emerged almost a century later, with the release of commercial liquid systems. These systems provided significant improvements over solid media (shorter turn-around time for results and an increased yield in diagnosis) but up to this day remain technically complex and costly.

The 1990s saw ground-breaking discoveries in molecular diagnostics, and the tuberculosis world started to benefit from rapid technologies to detect drug resistance. Molecular line probe assays, allowing a DST result within 24 hours for rifampicin or rifampicin plus isoniazid multidrug resistance, were approved by the

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World Health Organization (WHO) in 2008 [3]. Another breakthrough came in 2010 when the first automated, closed, molecular system simultaneously detecting tuberculosis and rifampicin resistance in less than 2 hours was released: the Xpert MTB/RIF assay, running on the GeneXpert system [4], developed through an innovative collaboration between academia (University of Medicine & Dentistry of New Jersey), industry (Cepheid Inc.) and FIND, with US governmental support.

WHO approval of the Xpert MTB/RIF assay in late 2010 and rapid global uptake [5], facilitated by updated WHO policy guidance in 2013 [6] stimulated unprecedented interest in the development of "rapid followers" as can be seen from the robust pipeline of new diagnostics [7]. Most impressive is the range of molecular technologies that could potentially—and in the short term—replace smear microscopy which, despite its shortcomings, remains the cornerstone of tuberculosis diagnosis in all but the wealthiest countries.

Anticipated improvements in the Xpert MTB/RIF assay [8] and successful validation of other diagnostics in the pipeline may allow a future without (or at least much fewer) sophisticated and expensive containment laboratories for conventional culture and DST. For tuberculosis drug resistance testing, sequencing technology is increasingly playing an important role in resolving discordant results between genotypic and phenotypic tests and emerging data seem to suggest that molecular testing may become the new "gold standard" for DST in the not too distant future. This will, however, require that sequencing technology be brought closer to point-of-care and become affordable to resource-limited countries.

Less robust [7] is the pipeline for non-sputum-based diagnostic products and biomarker-based triage tests that can be used at point-of-care. This will require a breakthrough in biomarker discovery, and the conduct of well-designed trials to optimize screening and diagnostic algorithms. Urgent yet strikingly absent from the pipeline [7] are biomarker-based tests for monitoring treatment, alternatives to culture as primary endpoint for cure in clinical trials, and tests to identify people with latent tuberculosis infection who are at the highest risk of progressing to tuberculosis disease.

The WHO post-2015 End Tuberculosis strategy and its related targets adopted by the World Health Assembly in May 2014 call for early diagnosis of tuberculosis including universal DST and systematic screening of contacts and high-risk groups [9]. The initiatives outlined in this special supplement address several of the essential components necessary for accelerated discovery and innovation of new tuberculosis diagnostics: a) defining the needs for next-generation assays; b) developing target product profiles; c) collecting data on resistance-associated mutations; iv) assessing the market potential for new tuberculosis diagnostics; v) modelling cost and affordability of next-generation assays and diagnostic algorithms. In addition, well-designed validation and field trials of new diagnostics in intended settings of use will be essential to allow rapid policy development according to WHO criteria [5].

All the components outlined in this supplement are also crucial for the introduction of new tuberculosis drugs and expected new tuberculosis regimens over the next few years. For new regimens in particular, rapid identification of drug resistance in individual patients will be key to ensure optimal outcomes and prevent amplification of resistance. A major need, therefore, is to align diagnostic test development with anticipated novel tuberculosis regimens in synergised research efforts [10]. Such efforts could greatly benefit from much closer collaboration of researchers, test developers, technical agencies, funders and end-users (eg, country Ministries of Health) of sequence-based technologies, from trials that combine new diagnostics and treatment (drugs and regimens) in innovate designs, and from links with ongoing global initiatives such as the WHO drug resistance surveillance project [11]. Of crucial importance is accelerated research to evaluate the clinical prognostic value of drug resistance mutations, especially for second-line and new anti-tuberculosis drugs.

The blueprint for collective and consensus-driven tuberculosis diagnostic test development outlined in this special supplement is based on strong collaborations between industry, academia and technical/donor agencies, and end-users—which bodes well for diagnostic development in the future. These efforts deserve to be supported and the funding constraints [12] should be urgently addressed in equally innovative approaches.

Koch's discovery of the tubercle bacillus revolutionized the management of tuberculosis in the 19th century. Pursuing new innovations with the same zeal as Ehrlich did of the humble microbiology stain by Koch in 1882, and working in collaborative partnerships such as the the the one outlined in this supplement will ensure that new innovations for tuberculosis today do not take a century to reach those in need. As René and Jean Dubos wrote in 1952 [13]: "In science the credit goes to the man who convinces the world, not to the man to whom the idea first occurs." The same holds true on World Tuberculosis Day 2015 as we prepare for a future without tuberculosis.

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