Microbiology Spectrum
American Society for Microbiology Press

Tuberculosis Diagnostics: State of the Art and Future Directions

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ABSTRACT Rapid and accurate diagnosis is critical for timely initiation of anti-tuberculosis (TB) treatment, but many people with TB (or TB symptoms) do not have access to adequate initial diagnosis. In many countries, TB diagnosis is still reliant on sputum microscopy, a test with known limitations. However, new diagnostics are starting to change the landscape. Stimulated, in part, by the success and rollout of Xpert MTB/RIF, an automated, molecular test, there is now considerable interest in new technologies. The landscape looks promising with a pipeline of new tools, particularly molecular diagnostics, and well over 50 companies actively engaged in product development, and many tests have been reviewed by WHO for policy endorsement. However, new diagnostics are yet to reach scale, and there needs to be greater convergence between diagnostics development and the development of shorter TB 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 latent TB progression. Increased investments are necessary to support biomarker discovery, validation, and translation into clinical tools. While transformative tools are being developed, high-burden countries will need to improve the efficiency of their health care delivery systems, ensure better uptake of new technologies, and achieve greater linkages across the TB and HIV care continuum. While we wait for next-generation technologies, national TB programs must scale up the best diagnostics currently available, and use implementation science to get the maximum impact.

INTRODUCTION

Despite the progress made in global tuberculosis (TB) control, TB remains a major global health problem, and drug-resistant TB is a growing threat (1). Early diagnosis of TB including universal drug susceptibility testing (DST), and systematic screening of contacts and high-risk groups are key components of the End TB Strategy by WHO and partners (2).

Rapid, accurate diagnosis is critical for timely initiation of anti-TB treatment, but many people with TB (or TB symptoms) do not have access to adequate initial diagnosis. For example, 37% of the 9.6 million new cases globally are either undiagnosed or not reported. These "missing" 3.6 million people with TB are at the root of ongoing TB transmission, including of multidrug-resistant TB (MDR-TB) (1). Seventy-five percent of the 480,000 cases of MDR-TB are either not detected or not reported (1). Even among previously treated patients at risk of drug resistance, 40% were not tested for drug resistance, and 50% of TB patients have no documented HIV test result (1).

In this article, we provide an overview of current diagnostics for active TB and drug susceptibility testing, and review the unmet needs and gaps. Latent TB diagnostics are covered elsewhere (69). We also describe the pipeline of new diagnostics, and review lessons learned from implementation research on how to deploy new tools for maximum impact.

Received: 4 May 2016, **Accepted:** 4 July 2016,
Published: 21 October 2016

Editors: William R. Jacobs Jr., Howard Hughes Medical Institute, Albert Einstein School of Medicine, Bronx, NY 10461; Helen McShane, University of Oxford, Oxford OX3 7DQ, United Kingdom; Valerie Mizrahi, University of Cape Town, Rondebosch 7701, South Africa; Ian M. Orme, Colorado State University, Fort Collins, CO 80523

Citation: Pai M, Nicol MP, Boehme CC. 2016. Tuberculosis diagnostics: state of the art and future directions. *Microbiol Spectrum* 4(5):TBTB2-0019-2016. doi:10.1128/microbiolspec.TBTB2-0019-2016.

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CURRENT DIAGNOSTICS FOR ACTIVE TB

Currently, there are three main validated methods for the detection of active TB: microscopy, nucleic acid amplification tests (NAATs), and cultures. In addition, antigen detection tests are commercially available with limited WHO endorsement. For screening of active TB, imaging with chest X ray is a widely used method and may become of increased utility with the emergence of digital radiology and computer-aided interpretation (3). Table 1 shows the technologies that have undergone WHO review, in each category.

Smear Microscopy

Stains that are taken up by the lipid-rich cell wall of *Mycobacterium tuberculosis* resist decolorization with acid-containing reagents. Acid-fast organisms can then be visualized on microscopic examination of smears prepared from sputum or other biological specimens. The most widely used method in low-resource settings involves examination of Ziehl-Neelsen-stained slides under light microscopy. However, fluorescent microscopy, with stains such as Auramine, is 10% more sensitive and permits more rapid screening (at lower magnification) of large numbers of smears (4). Until recently, fluorescent microscopy was relatively expensive, with costly microscopes requiring frequent maintenance (e.g., bulb changes). The replacement of conventional fluorescent light sources with light-emitting diodes (LED fluorescent microscopy) has substantially reduced cost, power (LED microscopes can be battery powered), and maintenance requirements, and has eliminated the need for a darkroom, while retaining sensitivity (5).

The benefit of concentrating sputum prior to microscopy, by centrifugation or sedimentation of sputum that has been liquefied using bleach or NaOH (with or without *N*-acetyl-l-cysteine [NALC]) remains unclear. A systematic review demonstrated that concentration, on average, increased sensitivity, with no loss in specificity, but results varied widely between studies (6); in the case of LED microscopy, concentration appears to significantly decrease sensitivity (5).

The major limitation of smear microscopy is lack of sensitivity, which varies widely (20 to 80%) and is particularly poor in patients with paucibacillary TB including children, patients with extrapulmonary TB, or those who are HIV coinfected. It is estimated that 5,000 to 10,000 bacilli are required per milliliter of sputum for a positive direct (unconcentrated) smear. Specificity is likely to vary considerably depending on the local prevalence of infections with nontuberculous mycobacteria. In regions with a high incidence of tuberculosis, specificity of smear microscopy is high (95 to 98%), although there is some evidence that concentration by centrifugation is associated with variable reduction in specificity.

In summary, the key WHO recommendations (5) for smear microscopy are:

- LED microscopy should replace conventional fluorescent and light microscopy.
- There is insufficient generalizable evidence that microscopy of concentrated sputum specimens provides results that are superior to direct smear microscopy.

TABLE 1 Technologies reviewed by WHO for TB case detection

Year	Method	Technology reviewed by WHO
2007	Culture (growth-based)	Commercial liquid culture and rapid speciation strip tests
2010	Microscopy	LED microscopy
2010	NAAT	Xpert MTB/RIF
2016	Antigen detection test	Urine LAM rapid test
2016	NAAT	Loop-mediated amplification test (LAMP)

Commercial Liquid Culture and Rapid Speciation Strip Tests

Mycobacterial culture on solid agar (e.g., Lowenstein-Jensen [LJ]) or in liquid culture (e.g., Mycobacterial Growth Indicator Tube [MGIT; Becton Dickinson, Franklin Lakes, NJ] or BacT/ALERT MB [bioMérieux, Durham, NC]) remains the gold standard test for diagnosis of tuberculosis. A culture isolate of *M. tuberculosis* is still currently required for detailed DST and for genotyping to identify transmission events or outbreaks. Solid culture is less expensive than liquid culture and less prone to contamination by other bacteria or fungi, but liquid culture is faster, more sensitive (10% increased case detection), and convenient (growth is detected automatically by monitoring fluorescence) (7).

Samples that are contaminated with normal flora (such as sputum) must first undergo decontamination (typically using NaOH together with NALC), which kills rapidly growing bacteria and fungi, but which has a limited effect on mycobacterial viability. Importantly, high concentrations or prolonged exposure of mycobacteria to NaOH will reduce recovery, and so there is a fine balance between overdecontamination (which reduces the yield of mycobacterial culture) and under-

decontamination (which leads to failed cultures because of high rates of bacterial or fungal overgrowth).

Cross contamination occurs when *M. tuberculosis* from one sample or culture is carried over, during batch processing, to another sample. To minimize this risk, cultures should be manipulated in biosafety cabinets separate from those used for specimen processing, specimens with high bacterial load (smear-positive samples) should be processed before those with lower loads (smear-negative samples, samples from children), and single-aliquot reagents should be used.

There are several constraints to widespread implementation of mycobacterial culture, including the need for infrastructure and maintenance to support the appropriate level of biosafety, uninterrupted power supply, training, rapid transport of samples to the laboratory (maximum of 4 days if samples are refrigerated), and cost (7).

In order to take advantage of the more rapid turnaround of liquid culture, rapid identification of positive cultures should be used. Biochemical testing has largely been replaced by molecular or immunochromatographic lateral flow testing. High-throughput laboratories may be able to run frequent batches of line probe assays (see "Current diagnostics for Drug-Resistant TB," below), which confirm the identification as *M. tuberculosis* complex. Alternatively, lateral flow assays incorporating monoclonal antibodies against the *M. tuberculosis* protein MPB64 have been demonstrated to be highly sensitive and specific for *M. tuberculosis* complex, and are simple, rapid, and inexpensive (8).

In summary, the key WHO recommendations (7) on mycobacterial cultures are:

- Liquid culture is feasible for implementation in lower-income settings.
- Liquid culture has a higher rate of mycobacterial isolation and a shorter time to detection compared with solid culture.
- Rapid differentiation of *M. tuberculosis* from other acid-fast organisms recovered in culture is essential.

Xpert MTB/RIF

This cartridge-based molecular assay enables rapid detection of *M. tuberculosis* and simultaneous identification of rifampin resistance directly from clinical specimens, with minimal operator dependence. Sputum (or other suitable sample) is liquefied and inactivated using a fixed ratio of NaOH and isopropanol-containing sample reagent. The liquefied sample is then added to a cartridge where the sample is automatically filtered (to capture *M. tuberculosis* bacilli), sonicated (to release bacterial DNA), and hemi-nested real-time PCR is performed (9). The PCR targets an 81-bp region of the *rpoB* gene of *M. tuberculosis* where more than 95% of mutations associated with rifampin resistance occur. Five molecular probes are designed to bind to the wild-type (sensitive) gene of *M. tuberculosis*; binding is detected by fluorescent signals from each of these probes. Signal from at least two of these probes indicates the presence of *M. tuberculosis*, while delay in binding, or failure to bind, of at least one probe indicates rifampin resistance (9).

The limit of detection of the Xpert MTB/RIF assay in spiked sputum samples has been measured at 131 bacilli per ml of sputum (10). Pooled estimates of sensitivity and specificity of the assay for tuberculosis detection from studies of patients with presumed pulmonary tuberculosis are 89% and 99%, respectively (11). As with smear microscopy, sensitivity is lower in patients with HIV infection (79%) and in children (66%) (12). For extrapulmonary samples, sensitivity varies with sample type. Sensitivity is highest for lymph node biopsies/aspirates and cerebrospinal fluid but poor for pleural fluid (13, 14).

An important limitation of Xpert MTB/RIF is its inability to distinguish between live and dead bacilli. The assay may remain positive even after treatment completion and should not be used to monitor response to treatment (15). Constraints to widespread rollout include cost, need for continuous power supply, sensitivity to high temperatures, and assay throughput. A more sensitive assay, Xpert MTB/RIF Ultra, is currently under clinical evaluation, and is likely to be as sensitive as liquid culture. A more robust, point-of-care, portable, battery-operated GeneXpert platform is also being developed. This device, called GeneXpert Omni, will be available in 2017.

In summary, the key WHO recommendations (16) on Xpert MTB/RIF are:

- Xpert MTB/RIF should be used as the initial diagnostic test in adults or children suspected of having MDR-TB or HIV-associated TB.
- Xpert MTB/RIF may be used as the initial diagnostic test in all adults or children suspected of having TB (conditional recommendation acknowledging resource implications).
- Xpert MTB/RIF should be used as the initial diagnostic test for cerebrospinal fluid specimens from patients suspected of having TB meningitis.

- Xpert MTB/RIF may be used as a replacement test for usual practice for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB.

Loop-Mediated Amplification Test

Apart from Xpert MTB/RIF, several other NAATs for TB are at various stages of development. The TB-Loop-Mediated Amplification Test (LAMP) assay (Eiken Chemical Co., Japan) is based on an isothermal amplification protocol (using a simple heating block) and produces a result that can be seen with the naked eye. It therefore offers advantages in terms of cost and suitability for implementation in peripheral settings (17). However, recent data suggest that to achieve acceptable performance of LAMP at the microscopy center level, significant training and infrastructure requirements are necessary (17).

An earlier version of LAMP was reviewed by WHO in 2013 (18). The sensitivity of LAMP was found to be good for smear-positive samples (97%) and lower for smear-negative samples (53 to 62%). Specificity was suboptimal (95 to 97%); low specificity may be due to failure to follow the manufacturer's recommendations precisely. Low specificity may result in unacceptably low positive predictive value of a positive test in low TB prevalence countries. An updated WHO policy on LAMP, based on an improved assay with new evidence, is expected in 2016.

Urine Lipoarabinomannan Rapid Test

An alternative to detection of whole *M. tuberculosis* bacilli or DNA is detection of structural or secreted *M. tuberculosis*-specific biomolecules in patient samples. Lipoarabinomannan (LAM) is a component of the cell wall of *M. tuberculosis* that may be found in urine of patients with TB. It is not clear whether circulating LAM is filtered by the glomeruli (this may be less likely because LAM typically circulates in an immune complex or associated with high-density lipoprotein carrier molecules) (19) or whether the presence of LAM in urine is due to (subclinical) urinary tract infection with *M. tuberculosis* (20). The initial enzyme-linked immunosorbent assay-based test for LAM has now been replaced with a lateral flow assay suitable for implementation at, or close to, the point of care.

Urine LAM testing lacks sensitivity for diagnosis of TB in HIV-uninfected patients, and should only be used for diagnosis of HIV-associated TB in patients with low CD4 counts (<100 cells/μl), or HIV-infected patients who are seriously ill. Even in this patient group, sensitivity is suboptimal (pooled sensitivity 56%) (21). Reported specificity of the test varies; however, this is likely due to differences between studies in the effort taken to establish a reference standard diagnosis. When extensive investigation is done for TB, and when band intensity of grade 2 on the test strip is used as a cut point for a positive result, it appears that specificity of the LAM test is high (22). The test is not able to distinguish between infection with *M. tuberculosis* and other mycobacterial species; however, the positive predictive value is likely to be high in countries endemic for TB. A positive test is therefore sufficient grounds to start treatment for TB in such countries; however, a negative test cannot be used to rule out TB. While the clinical applicability of this test may be limited, LAM testing and rapid initiation of TB treatment among HIV-infected inpatients suspected to have TB in high-burden countries may reduce early mortality (23).

In summary, the key WHO recommendations (21) on LAM are:

- LAM testing should only be used to assist in the diagnosis of TB in persons with HIV infection with low CD4 counts (<100 cells/μl) or HIV infected patients who are seriously ill.
- LAM testing should not be used as a screening test for TB.

CURRENT DIAGNOSTICS FOR DRUG-RESISTANT TB

Currently, DST is performed using either phenotypic methods or genotypic methods. Table 2 shows the technologies that have undergone WHO review in each category.

Phenotypic Tests for DST

Methods used for phenotypic DST include the absolute concentration, resistance ratio, or proportion methods.

TABLE 2 Technologies reviewed by WHO for drug-susceptibility testing

Year	Method	Technology reviewed by WHO
2007	Phenotypic	Commercial liquid culture and DST
2008	Genotypic	Molecular LPAs for first-line anti-TB drug resistance detection
2010	Phenotypic	Selected noncommercial DST methods (MODS, CRI, NRA)
2010	Genotypic	Xpert MTB/RIF
2016	Genotypic	Molecular LPAs for second-line anti-TB drug resistance detection

Testing on solid agar using the proportion method is still regarded as the reference standard method. This is performed by counting the number of *M. tuberculosis* colonies that grow on agar without antibiotics compared with agar in which a critical concentration of antibiotic has been incorporated; if the number of colonies on antibiotic-containing media is >1% of that on the antibiotic-free media, the isolate is regarded as resistant. The critical concentration is primarily derived by epidemiological cutoff, as the concentration of antibiotic that best discriminates between a population of wild-type bacteria (which have never been exposed to antibiotic) and resistant bacteria (which have persisted in the presence of treatment). While for some antibiotics there is an identifiable concentration that discriminates well between these groups, for some others (e.g., ethambutol) there is considerable overlap between wild-type and resistant organisms, and this limits the applicability of phenotypic DST for these drugs.

Commercial Liquid Culture-Based DST

Commercial automated liquid culture systems (e.g., MGIT, above) use a modification of the proportion method and offer reliable results for isoniazid and rifampin, as well as for fluoroquinolones, aminoglycosides, and polypeptides. Testing for resistance to other first-line (ethambutol and pyrazinamide) and second-line drugs is less reliable and reproducible; automated liquid systems are recommended for testing (24).

A limitation of many current commercial systems is the inclusion of only one (or sometimes two) critical concentrations of each antibiotic. The result given is qualitative rather than a semiquantitative minimum inhibitory concentration (MIC). This may be of relevance; for example, recent data suggest that clinically relevant "low-level" resistance to rifampin is missed by testing only one concentration of rifampin (1 μg/ml) (25). An alternative approach is to perform detailed MIC testing for specific antibiotics, particularly in difficult-to-treat highly resistant cases, where individually tailored drug treatment may be required, for example, using the commercial Sensititre *M. tuberculosis* MIC Plate (ThermoFisher, Waltham, MA).

In summary, the key WHO recommendations (7, 24) on liquid culture-based DST are:

- As a minimum, national TB control programs should establish laboratory capacity to detect MDR-TB.
- Automated liquid systems and molecular line probe assays (see "Genotypic Tests for DST," below) for first-line DST are recommended as the current gold standard.
- DST for aminoglycosides, polypeptides, and fluoroquinolones has been shown to have relatively good reliability and reproducibility.

Noncommercial DST Methods

Several noncommercial methods have been developed as alternatives to the automated commercial systems for DST. These methods may be less expensive, but are generally less well standardized, are highly operator dependent, and may have local variation in methodology. They therefore need to be supported by strong quality assurance mechanisms, and should be performed only in reference, centralized laboratories (26). These methods include:

- Microscopic observation of drug susceptibility (MODS), which relies on microscopic observation of microcolonies of *M. tuberculosis* in liquid media (with and without antibiotics). Microtiter plates may be inoculated with sputum specimen (direct testing) or cultured isolates (indirect testing).
- Nitrate reductase assay (NRA), which is based on colorimetric change in solid agar caused by reduction of nitrate by *M. tuberculosis*, and is suitable for direct or indirect testing.
- Colorimetric redox indicator (CRI) methods, which are based on color change due to reduction of an indicator dye that is added to liquid media containing viable *M. tuberculosis* that has been exposed to antibiotics (indirect testing only).

There is insufficient evidence to recommend other noncommercial methods, such as phage-based assays and thin-layer agar for use (26). In summary, key WHO recommendations (26) on noncommercial DST methods are:

- MODS, CRI, and NRA methods may be used under clearly defined program and operational conditions, in reference laboratories, and as an interim solution while capacity for genotypic or automated liquid culture is being developed.

Genotypic Tests for DST

The genetic basis for acquired drug resistance in *M. tuberculosis* is change (single-nucleotide polymorphisms, deletions, insertions) in the mycobacterial chromosome. Such changes may be detected by interrogating the relevant gene sequence, either directly by DNA sequencing, or indirectly, using probe-based methods or methods that rely on the effect of such mutations on the melting temperature of double-stranded DNA.

Line Probe Assays for Detection of Resistance to First-Line Anti-TB Drugs (Isoniazid and Rifampin)

Line probe assays (LPAs) (e.g., GenoType MTBDR*plus*, Hain Lifescience, Nehren, Germany; and NTM+MDRTB Detection Kit 2, Nipro Corporation, Japan) identify drug-resistance mutations by detecting the binding of PCR-amplified fragments of *M. tuberculosis* DNA to probes targeting the most common mutations conferring resistance to isoniazid and rifampin or to wild-type probes. Resistance is identified by detecting hybridization of DNA from the patient isolate to a mutant (resistant) probe and/or by detecting failure of hybridization to a wild-type (sensitive) probe. Mutations in the *inhA* promoter and *katG* regions (responsible for most isoniazid resistance) and the rifampin-resistance-determining region of the *rpoB* gene (responsible for most rifampin resistance) are targeted. Molecular testing using LPA is significantly more rapid than phenotypic DST, presents lower biosafety risk, and increases throughput.

In meta-analysis, the pooled sensitivity of Hain MTBDR*plus* was 98% (95% CI, 96 to 99%) for detection of resistance to rifampin but more variable for isoniazid (pooled sensitivity 84%; 95% CI, 77 to 90%). Specificity for both was excellent (99%) (27).

LPA testing may be done on cultured isolates or directly from smear-positive sputum samples. Limited data (28) suggest that LPA can also be done directly from smear-negative sputum samples (i.e., for both diagnosis of tuberculosis as well as resistance testing); however, there are insufficient data to recommend the use of LPA in this patient group. A significant limitation of LPA is that the test requires "open" manipulation of PCR amplicons, so the risk of cross contamination between samples is high. Meticulous attention to unidirectional workflow, well-trained staff, and a strong quality assurance program are required to reduce this risk.

In 2008, WHO endorsed the use of Version 1 of the GenoType MTBDR*plus* assay for rapid detection of isoniazid and rifampin resistance on smear-positive samples. In 2015, WHO published an update of the LPA policy where GenoType MTBDR*plus* Version 2 and the Nipro Corporation (Japan) NTM+MDRTB Detection Kit 2 were endorsed. Either tool can be used to detect TB and to genotype alleles that confer resistance to rifampin and isoniazid from either smear-positive sputum samples or from culture-derived isolates.

In summary, the key WHO recommendations (29) on molecular LPAs are:

- LPAs are validated for direct testing of sputum in smear-positive specimens and on isolates of *M. tuberculosis*. They are not recommended for use on smear-negative samples.
- Adoption of LPAs does not eliminate the need for conventional culture and DST capability (for diagnosis of patients with smear-negative TB and for further DST for patients with MDR-TB).
- Appropriate laboratory infrastructure and appropriately trained staff are necessary to ensure adequate precautions for biosafety and prevention of contamination.

Xpert MTB/RIF for Rifampin Resistance

The principle of the Xpert MTB/RIF assay for detection of rifampin resistance has been described in "Xpert MTB/RIF," see above. The pooled sensitivity for detection of resistance to rifampin in meta-analysis was 94% and specificity was 98% (11). The interpretation of these findings is somewhat complicated by different assay versions being tested in different studies; however, in countries with a low prevalence of rifampin resistance, the positive predictive value of Xpert MTB/RIF for rifampin resistance is likely to be relatively low. A rifampin-resistant Xpert MTB/RIF result should therefore be confirmed with a second (different) test. Furthermore, the correlation between genotypic and phenotypic testing is sometimes complex (30). For example, as described above ("Commercial Liquid Culture-Based DST"), liquid culture-based phenotypic tests may miss low-level rifampin resistance, but these are usually detected by genotypic (Xpert MTB/RIF or LPA) testing (25). The reverse may also be true; Xpert MTB/RIF may miss some locally prevalent rifampin resistance-conferring mutations, which are detectable by phenotypic testing. Detailed understanding of the limitations of the various testing methods is required, as is knowledge of the local distribution of resistance-conferring mutations.

LPAs for detecting resistance to second-line anti-TB drugs

At present, the reference standard for DST for second-line TB drugs is phenotypic testing (liquid or agar proportion). However, in patients in whom a rapid diagnosis of rifampin-resistant TB has been made by molecular testing (Xpert MTB/RIF or LPA), there is often considerable delay before results of phenotypic tests are available. Uncertainty on the most appropriate treatment regimen may delay effective treatment and result in amplified resistance (acquisition of resistance to additional drugs). Rapid genotypic tests for resistance to second-line drugs may reduce this delay. Second-line LPA (MTBDR*sl*, Hain

Lifescience) provides information on resistance to injectable drugs and fluoroquinolones. The current version of this assay includes *gyrA* and *gyrB* for detection of resistance to fluoroquinolones and *rrs* and *eis* for detection of resistance to injectable drugs. The previous version of this assay (which did not include *gyrB* nor *eis* but included *embB* for ethambutol resistance) was estimated to have a pooled sensitivity of 83% for detection of resistance to fluoroquinolones and 77% for injectable resistance (31) (this varied by specific drug, since there is incomplete cross-resistance among injectable drugs). Specificity for both was good (>98%). Therefore, this test is useful as a rule-in test for extensively drug-resistant (XDR) or pre-XDR tuberculosis, but, because of suboptimal sensitivity, it cannot be used to completely rule out resistance. Detailed understanding of the local distribution of drug-resistance mutations is required to interpret results of this assay in the local context. In 2016, WHO published an updated policy on second-line LPA (32).

In summary, the key WHO recommendations (32) on second-line molecular line probe assays are:

- For patients with confirmed rifampin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones
- For patients with confirmed rifampin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the second-line injectable drugs

Unmet Needs and Gaps

A recently published study of various stakeholders helped establish the most important unmet needs, and helped identify tools that are of highest importance. Kik and colleagues conducted a priority-setting exercise to identify the highest-priority tests for target product profile (TPP) development and investment in research and development (33). For each of the potential TPPs, 10 criteria were used to set priorities, including prioritization by key stakeholders (e.g., National TB Program [NTP] managers), potential impact of the test on TB transmission, morbidity and mortality, market potential and implementation, and scalability of the test. Based on this analysis, the following were identified as the highest priorities (33):

1. A point-of-care sputum-based test as a replacement for smear microscopy;
2. A point-of-care, non-sputum-based test capable of detecting all forms of TB;
3. A point-of-care triage test, which should be a simple, low-cost test for use by first-contact health care providers as a rule-out test;
4. Rapid DST at microscopy center level.

The second and third tests are especially critical also for improved diagnosis in children, who make up an estimated 10% of the global TB burden (34), people living with HIV, and those who have extrapulmonary TB. In the longer term, TB elimination cannot be achieved without identifying those with latent infection who are at the highest risk of progressing to active TB disease. A new test for cure will also be needed to monitor TB treatment (35).

Given the variety of unmet needs and the diversity of sites where testing can occur, it is important for product developers to have access to: (i) a clearly identified list of diagnostics that are considered high priority by the TB community; (ii) well-developed, detailed TPPs for priority diagnostics, based on a consensus-building process; and (iii) up-to-date market size estimations for the priority TPPs.

In 2014, WHO published a consensus document with TPPs for priority diagnostics (36), with elaborations (37, 38). A series of new publications have summarized the served available market in select countries, and the data suggest a sizeable annual TB diagnostics market worth an estimated US$ 480 million in Brazil, China, India, and South Africa combined (39–42). Market projections for future TB diagnostics have also been made (43). These market analyses will, hopefully, encourage greater investments in new product development. All these resources are now available at www.tbfaqs.org.

Pipeline of Future Diagnostics

Figure 1 shows the pipeline of new TB diagnostics, classified by level of complexity and stage of development. At first glance, the pipeline appears well populated. Most products in the pipeline are molecular based, making use of the only proven TB bacterial nucleic acid sequences. Although these tests hold promise for smear replacement and expanded DST, they are unlikely to meet affordability and ease-of-use requirements for integration into primary care. To meet these needs, we need new biomarkers and approaches. Although investment and activity in biomarker research has increased, translation from basic biomarker discovery to clinical applications has been poor (35).

Biomarker discovery efforts focus on host and pathogen markers and we see promising leads in some of the biomarker classes shown in Fig. 2 (44–47). For example, improved detection of the lipoglycan biomarker

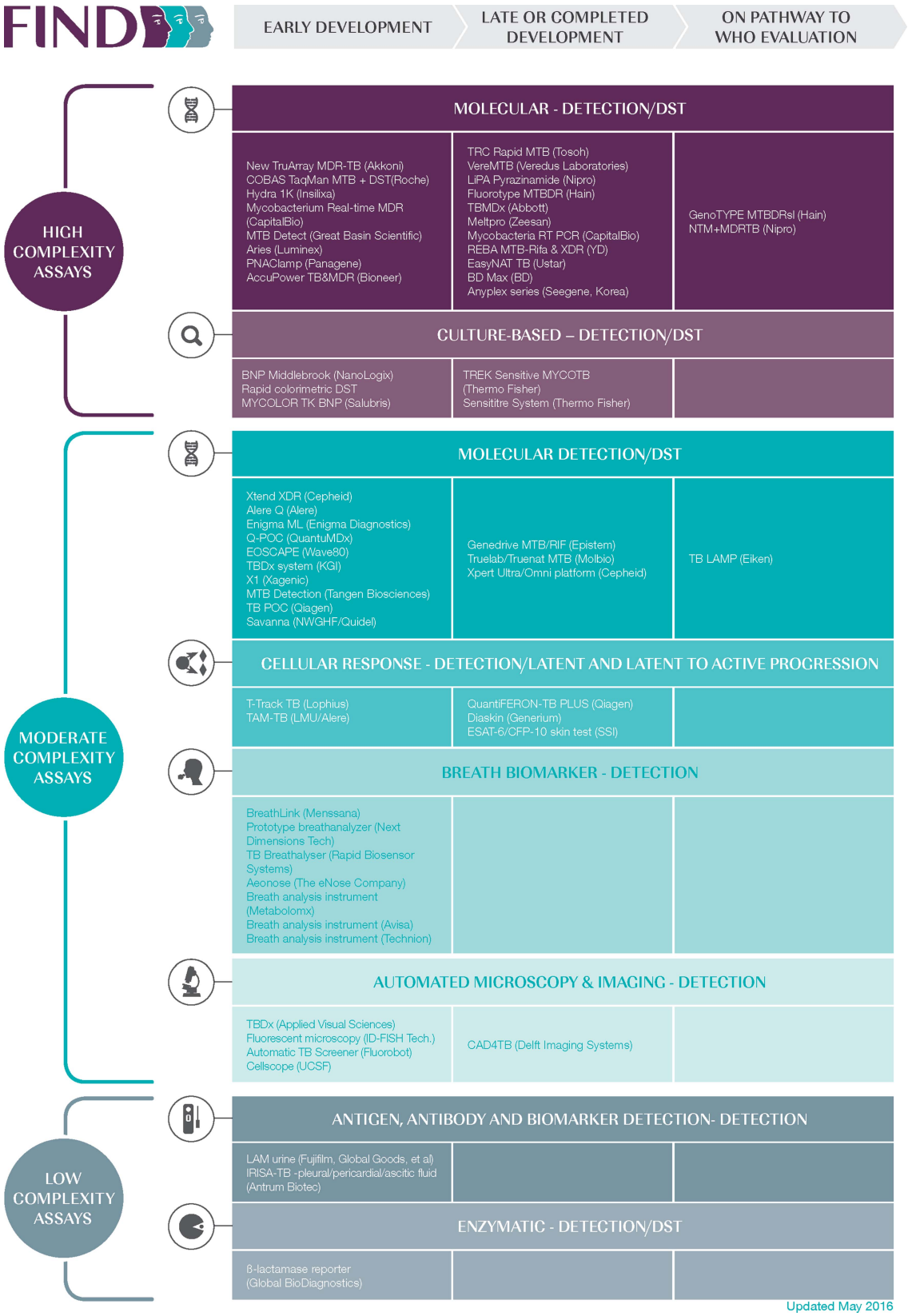


FIGURE 1 Pipeline of TB diagnostics (source: FIND, Geneva; www.finddx.org).

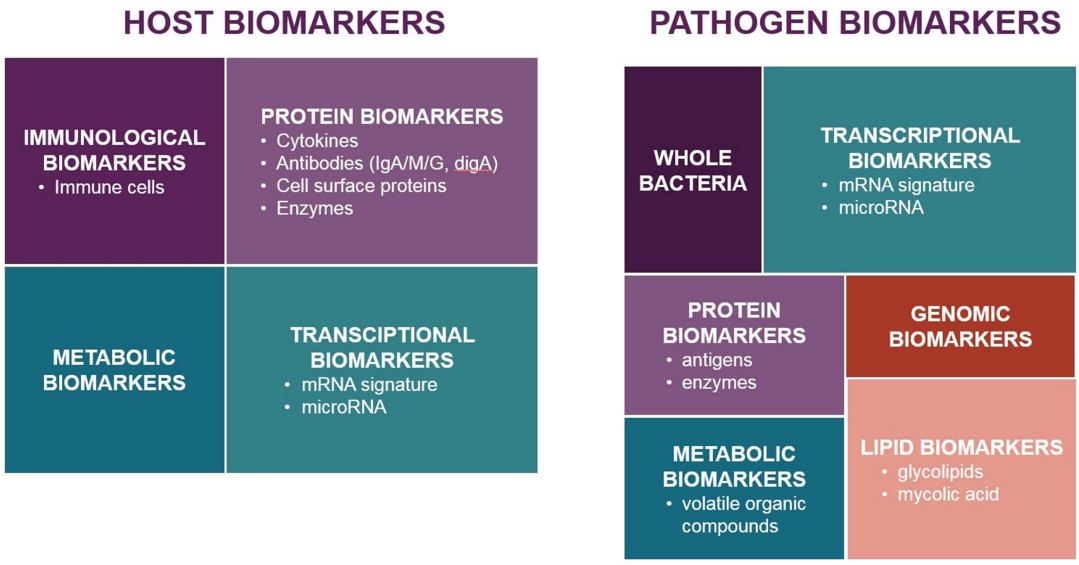


FIGURE 2 Classes of TB biomarkers under development and validation (source: FIND, Geneva; www.finddx.org).

lipoarabinomannan (LAM) could lead to a breakthrough in urine-based antigen detection (35). An area that gets a lot of attention is TB detection in breath to identify volatile organic compounds (VOCs) (48). Early indications suggest that prototypes fall short of required sensitivity and specificity, and lack independent evaluation. Serological tests detecting antibody responses such as lateral flow assays are appealing because of their simplicity, cost, and lack of specimen processing. However, existing serological assays have failed, and WHO has recommended against their use (49). Ongoing research might help overcome existing challenges. A lot of research is currently focused on host transcriptional markers, notably mRNA signatures to differentiate active from latent TB in both children and adults (44–47). Regardless of promise, none of these findings will yield a policy-endorsed product in the next 3 to 5 years.

In addition to rapid case detection, we need to identify new tools with expanded DST capabilities to help countries reach the post-2015 target of universal DST for all TB patients at the time of diagnosis. With the anticipated introduction of new TB drug regimens, we need to be able to test sensitivity to all critical regimen components (50). Many of the molecular assays in the pipeline aim to expand the drug menu. However, since drug resistance in *M. tuberculosis* can occur as a result of mutations in many different regions of the genome, targeted molecular testing for detection of drug resistance will always be constrained by the need for highly multiplexed assays (51).

In this context, next-generation sequencing tools are showing great promise and may become the method of choice for detailed DST of resistant isolates in the next 5 to 10 years (52, 53). While not WHO endorsed or yet widely used, feasibility has been shown, and the advantages of this approach are the ability to screen broadly for mutations conferring resistance to a range of different anti-TB drugs as well as obtain genotype information useful for tracking transmission and outbreaks (54). However, the paucity of good data on the correlation of mutations with phenotypic DST results and clinical outcomes, and the association with cross-resistance are preventing translation into routine use for clinical decision making. Before sequencing can become more widely used in high-burden settings, there is much work to be done to further simplify and automate procedures and equipment to reduce the need for highly skilled staff, notably improvements in specimen processing and software-based data interpretation support. In addition, the price will have to come down, although cost is becoming competitive with detailed DST (52).

While the TB diagnostics research and development (R&D) space has managed to attract over 50 companies and product developers, they will require technical and funding support to overcome the translational challenges shown in Table 3 (adapted from reference 55). While many manufacturers remain interested in the development of biomarker-based point-of-care tests, they face significant challenges with identification and validation. Many of the gaps in prioritized diagnostics will not be filled by the current pipeline, which is

TABLE 3 Translational challenges for developing innovative TB technologies that can meet the needs

Indication for testing	Currently used tools	Limitations of existing tools	Desirable new tools	Translational challenges for new tool development
Triage test to identify individuals with presumed TB who need confirmatory testing	1. TB symptoms (e.g., 2 weeks of cough) 2. Chest X rays	1. Symptoms lack sensitivity and specificity, especially in HIV-infected populations and children. 2. Chest X rays are sensitive, but not specific for TB.	A simple, low-cost triage test for use by first-contact health care providers as a rule-out test, ideally suitable for use by community health workers	Lack of validated biomarkers
Diagnosis of active pulmonary TB	1. Sputum smear microscopy 2. Nucleic acid amplification tests (NAATs) 3. Cultures	1. Smear microscopy lacks sensitivity and cannot detect drug resistance. 2. NAATs are expensive and not easily deployable at the peripheral level. 3. Cultures are expensive and require biosafety level 3 (BSL3) laboratories, and results take time.	A sputum-based replacement test for smear microscopy A non-sputum-based biomarker test for all forms of TB, ideally suitable for use at levels below microscopy centers	While several NAATs are being developed for microscopy centers, they will need to be evaluated in field conditions for policy. For the nonsputum TB test, the biggest challenge is lack of validated biomarkers.
Diagnosis of extrapulmonary (EPTB) and childhood TB	1. Smear microscopy 2. Nucleic acid amplification tests 3. Cultures	1. Children and patients with EPTB often do not produce sputum. Invasive samples are usually necessary. Microscopy lacks sensitivity and cannot detect drug resistance. 2. NAATs are expensive and not easily deployable at the peripheral level. Sensitivity in EPTB samples is low. 3. Cultures are expensive and require BSL3 laboratories, and results take time.	A non-sputum-based biomarker test for all forms of TB, ideally suitable for use at levels below microscopy centers	For the nonsputum TB test, the biggest challenge is lack of validated biomarkers.
Drug susceptibility testing	1. Nucleic acid amplification tests 2. Cultures	1. Current NAATs cannot reliably detect all mutations and sensitivity for drugs other than rifampin is poor. 2. Cultures are expensive and require BSL3 laboratories, and results take time. Reliability of phenotypic is poor for second-line drugs.	A new molecular DST for use at a microscopy center level, which can evaluate for resistance to rifampin, fluoroquinolones, isoniazid, and pyrazinamide, and enable the selection of the best drug regimen	Lack of good data on the correlation of mutations with phenotypic results and clinical outcomes and the association with cross-resistance. There is also a need to align emerging TB drug regimens with companion diagnostics.
Diagnosis of latent TB infection (LTBI)	1. Tuberculin skin test (TST) 2. Interferon-gamma release assays (IGRA)	Neither TST nor IGRA can separate latent infection from active disease. Neither test can accurately identify those at highest risk of progression to active disease.	A test that can resolve the spectrum of TB, and identify the subset of latently infected individuals who are at highest risk of progressing to active disease and will benefit from preventive therapy	Lack of validated biomarkers
Test of cure (treatment monitoring)	1. Serial smear microscopy 2. Serial cultures	1. Smears lack sensitivity, and cannot distinguish between live and dead bacilli. 2. Serial cultures are expensive and time consuming.	An accurate test for cure that can be used to make changes in management (e.g., changes in regimens, or DST)	Lack of validated biomarkers

Adapted from Pai (55).

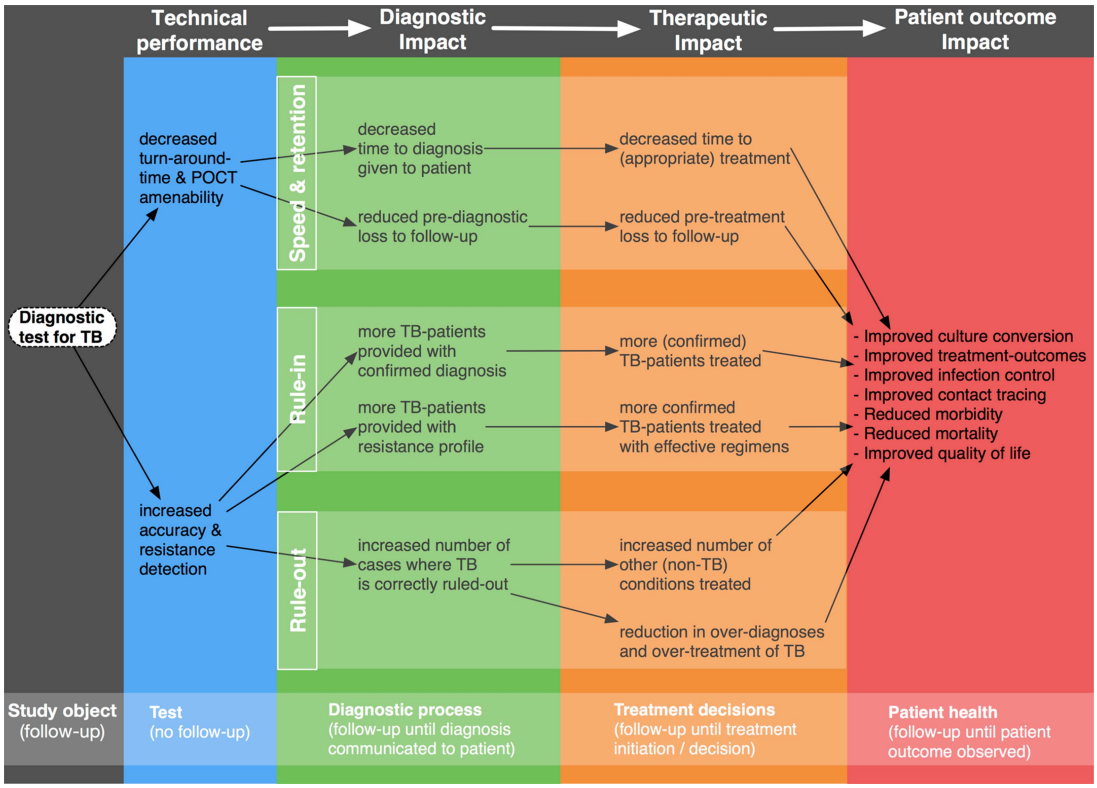
heavily weighted toward molecular smear replacement and DST, but not true point-of-care tests, ideally non-sputum-based, or tests to determine disease progression or cure. Increased investments are necessary to support biomarker discovery, validation, and translation into clinical tools. Unfortunately, a recent analysis of the TB R&D funding landscape by the Treatment Action Group showed a big gap between the investment needed and actual expenditure on diagnostics R&D (56). Donors and governments must work together to commit sustained funds toward agreed priorities in TB R&D, and the Stop TB Partnership will need to devise creative strategies to advocate for these funds.

How To Maximize the Impact of New Diagnostics, Based on Lessons from Xpert MTB/RIF Rollout

New TB technologies should have a significant impact on patient outcomes. However, as shown in Fig. 3 (57), the technical performance of tests is essential but, on its own, not sufficient. Operational weaknesses and underfunded TB programs hamper effective diagnostic uptake in many countries with high TB burdens. New tests must be paired with actions that ensure rapid—and, where possible, same-day—test results that drive appropriate and prompt clinical and treatment decisions. Good technologies and interventions must be effectively implemented to enable their full potential health impact.

In a recent article, Albert and colleagues reviewed the development, rollout, and impact of Xpert MTB/RIF, and described the lessons learned and identified areas for improvement with new tools (58). The global rollout of Xpert MTB/RIF has changed the TB diagnostic landscape. More than 16 million tests have been performed in 122 countries since 2011, and 6 million were performed in 2015 alone. This remains a small proportion of all TB tests conducted compared with conventional smear microscopy (some 30 million per year in the 22 high-burden countries) (59), and only eight countries have made it the initial diagnostic test for all people suspected of having TB or are in the process of doing so. However, it has become an important method for the detection of drug-resistant TB, which has seen a tripling in the number of cases detected globally since its introduction (1). The rollout has galvanized stakeholders, from donors to civil society, and paved the way for universal DST. It has also attracted new-product

FIGURE 3 How TB tests can potentially impact patient outcomes (source: Schumacher et al. [57]).



developers to tuberculosis, resulting in a robust molecular diagnostics pipeline.

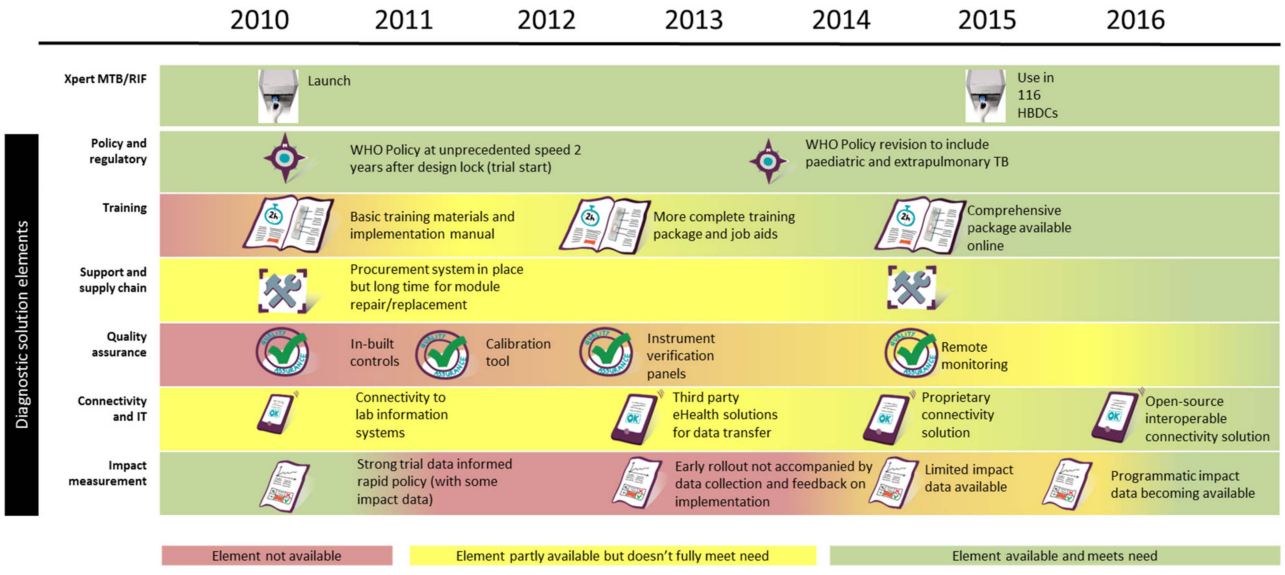
However, as the first widely used near-patient molecular platform in global health, the rollout of Xpert has highlighted major implementation gaps that have constrained scale-up and limited Xpert's impact on the outcomes of patients with drug-susceptible TB, although significant impact on time to treatment and mortality related to drug-resistant TB has been shown. The rollout has been hampered by high costs for underfunded programs in high-burden countries and lack of a complete diagnostic package for TB (Fig. 4) that includes comprehensive training, quality assurance, implementation plans, service and maintenance support, and impact assessment (58, 60). Clinical impact has been blunted by weak health systems, resulting in prolonged time to diagnosis and treatment (61, 62). In India, an average TB patient is diagnosed after a delay of nearly 2 months and after seeing three providers (63), and even though South Africa has scaled up rapid molecular testing, there are data showing long delays between sample collection and initiation of TB treatment (64, 65). In many countries the private sector plays a dominant role in TB control, yet this sector has limited access to subsidized Xpert MTB/RIF pricing (60).

A recent report called "Out of Step" by Doctors without Borders and the Stop TB Partnership surveyed eight countries with high TB burdens to see how existing TB policies and interventions were being implemented (59). This survey also found major implementation gaps in the diagnosis and treatment of MDR-TB. For example, in five of six countries providing data on drug susceptibility testing, fewer than 40% of previously treated cases were tested for first-line DST and fewer than 15% were tested for second-line DST. In four of eight surveyed countries, fewer than 75% of MDR-TB cases detected were enrolled in treatment. At the primary care level, TB testing is rare, even for patients with classic TB symptoms, and most patients are managed with repeated cycles of empirical broad-spectrum antibiotic therapies (66, 67). This has shown that an increased focus on same-day return of test results and effective linkage to care of diagnosed patients is required to maximize the potential impact of any new diagnostic tool.

In addition, in many countries the private sector plays a dominant role in TB control. New data suggest that Xpert MTB/RIF is very highly priced in the private sector in high-burden countries, and access is quite limited (60). The Initiative for Promotion of Affordable, Quality TB tests (IPAQT) intervention in India, which is bringing preferential pricing for new TB diagnostic tools to the private sector, is a first step in expanding access to rapid diagnosis in the places where many patients seek care (60).

In light of these lessons learned, the authors advocate for a comprehensive approach to the implementation of diagnostics, including pricing strategies for the private sector, broader health systems strengthening in preparation for new technologies, including greater linkages across the TB and HIV care continuum, and systematic and high-quality data collection from all programs.

FIGURE 4 Timeline of availability of required elements for Xpert MTB/RIF implementation (from reference 58 with permission).



While we wait for next-generation technologies, national TB programs must scale up the current best diagnostics, and use implementation science to get the maximum impact (68). Using Xpert MTB/RIF as the example, programs could achieve greater impact if they used Xpert as the initial test among all patients with suspected TB, reduced empiric treatment, and fostered decentralized implementation, guided by operational modeling to maximize cost effectiveness. In particular, programs can maximize impact by implementing Xpert in areas where routine diagnostic capacity is limited and by increasing access to private and informal sector providers who often see patients first.

CONCLUSIONS

Although TB diagnosis in many countries still relies on sputum microscopy, new diagnostics are starting to change the landscape. Stimulated, in part, by the success and rollout of Xpert MTB/RIF, there is now considerable interest in new technologies, but R&D funding commitments now need to catch up to the interest expressed. The landscape looks promising with a 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 the development of shorter TB 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 latent TB progression. Increased investments are necessary to support biomarker discovery, validation, and translation into clinical tools. In the meantime, high-burden countries will need to improve the efficiency of their health care delivery systems, ensure better uptake of new technologies, and achieve greater linkages across the TB and HIV care continuum. While we wait for next-generation technologies, national TB programs must scale up the current best diagnostics and use implementation science to get the maximum impact.

ACKNOWLEDGMENTS

M.P. has no financial or industry disclosures. He serves as a consultant to the Bill & Melinda Gates Foundation. He also serves on the Scientific Advisory Committee of FIND, Geneva. M.P.N. has received funding from the Foundation for Innovative New Diagnostics (Geneva, Switzerland) to do studies to assess the performance and effect of MTB/RIF. C.C.B. is employed by FIND, Geneva. FIND is a nonprofit organization that collaborates with several industry partners on the development, assessment, and demonstration of new diagnostic tests.

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