SUPPLEMENT ARTICLE

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

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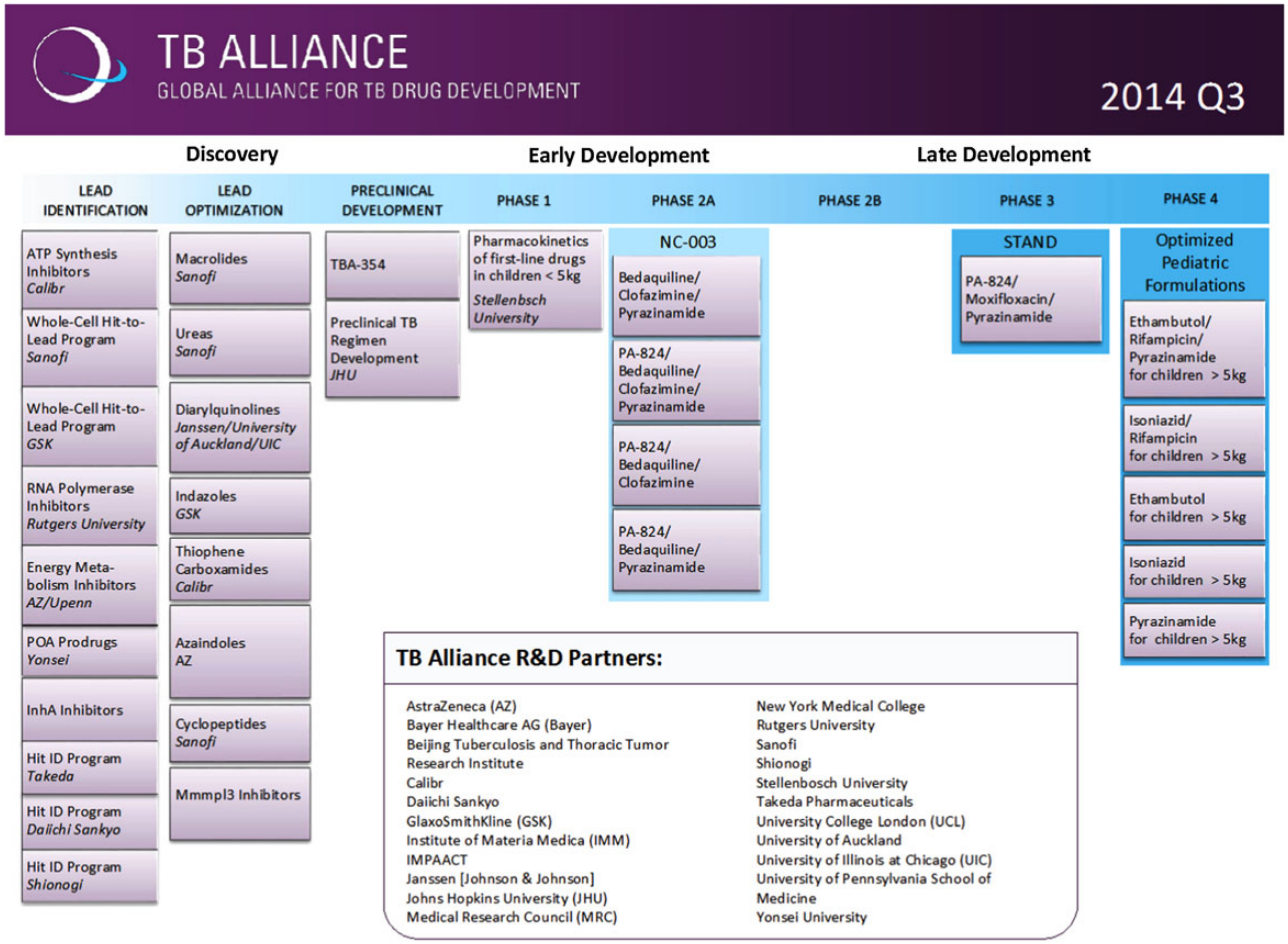

Figure 1. Tuberculosis Alliance pipeline. Reproduced with permission of the TB Alliance [13].

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 ≥1of 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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