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Alignment of new tuberculosis drug regimens and drug susceptibility testing: a framework for action

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New tuberculosis drug regimens are creating new priorities for drug susceptibility testing (DST) and surveillance. To minimise turnaround time, rapid DST will need to be prioritised, but developers of these assays will need better data about the molecular mechanisms of resistance. Efforts are underway to link mutations with drug resistance and to develop strain collections to enable assessment of new diagnostic assays. In resource-limited settings, DST might not be appropriate for all patients with tuberculosis. Surveillance data and modelling will help country stakeholders to design appropriate DST algorithms and to decide whether to change drug regimens. Finally, development of practical DST assays is needed so that, in countries where surveillance and modelling show that DST is advisable, these assays can be used to guide clinical decisions for individual patients. If combined judiciously during both development and implementation, new tuberculosis regimens and new DST assays have enormous potential to improve patient outcomes and reduce the burden of disease.

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Introduction

Patient care algorithms can be improved in two main ways: by rethinking and reorganising existing methods and technologies, and by introducing new technologies. In recent decades, national tuberculosis programmes have used existing technologies more effectively than in previous decades, achieving substantial results.[1] But further improvement is restricted by outdated and inadequate methods used to fight the epidemic: a vaccine with limited effectiveness; a drug regimen that is long and that places substantial demands on patients and health-care systems; and a diagnostic technique (smear microscopy) that detects only half of all cases and does not assess drug resistance of the infecting *Mycobacterium tuberculosis* strain.[2]

As efforts to improve these methods accelerate, investigators now have to consider how these various approaches will work together within a health system. Rapid development of resistance could occur if new drugs are added to failing regimens, or if combination regimens are used widely in populations that have substantial existing resistance to some of the drugs in those combinations. In some cases this resistance might leave only one effective drug in a regimen, increasing the chance of developing additional resistance and severely limiting the antimicrobial arsenal even further. Therefore, new tuberculosis regimens[3] cannot be introduced without development of drug susceptibility testing (DST) assays suited to the new regimens. DST can be used to monitor patterns of emerging drug resistance and to direct patients towards appropriate therapy, but careful analysis is needed to establish the optimum DST strategy for each new drug regimen and each different epidemiological context.

The primary backbone of tuberculosis treatment has not changed for decades; thus, susceptibility tests for

Key messages

- Advances in new drug regimens and diagnostics for tuberculosis, including drug susceptibility testing (DST), are exciting; however, strategies should be aligned to promote co-introduction for optimum results
- Tuberculosis treatment should ideally be based on full information about drug susceptibility of the infecting strain; however, at least in the short term and in resource-limited settings, less comprehensive DST might be more feasible or advisable in some countries; potential gains from DST should be balanced against costs, complexity, and predicted loss to follow-up
- DST and drug resistance surveillance are particularly important for existing and repurposed drugs, such as pyrazinamide and fluoroquinolones, that are being tested in first-line regimens and for which resistance already exists
- DST should be rapid to maximise patient retention and ensure prompt treatment with effective regimens, thus minimising the generation and spread of resistance; a rapid DST assay will probably need to detect molecular, rather than phenotypic, correlates of resistance
- To improve molecular tests, further research is needed to establish the genetic basis for resistance to existing and new drugs and to link each mutation with clinical effect; surveillance is needed to establish the background level of resistance
- This information can be used by modellers to assess the potential effectiveness of different scenarios of drug and diagnostic introduction; by product developers to better define product specifications; and by country programmes and providers to better assess whether, and how, to adopt new products

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additional drugs have not received much attention.[4] But regimens with new tuberculosis drugs will change priorities for DST and drug resistance surveillance. Resistance against drugs in new first-line regimens will be particularly important to test for, especially since existing tuberculosis drugs are easily available in the private sector—in large volumes, and with little or no regulation—in many high tuberculosis burden countries.[5]

Through the Tuberculosis Diagnostics Research Forum, several partners are working to ensure that the necessary DST assays are developed in time for co-implementation with new tuberculosis drug regimens. The aim is to develop a framework for designing DST for new regimens. Such DST should meet at least the same performance criteria as DST for existing first-line therapy. The ultimate goal is to have sufficient information—including prevalence of existing resistance—so that all patients with tuberculosis can be confident that their regimen will be safe and effective.

To reach these goals, translational science is needed to provide the basis for molecular diagnostics development. Furthermore, surveillance data and modelling are needed to design DST protocols and to guide decisions on regimen changes. And, in countries where the surveillance and modelling show that DST assays are necessary, development and use of these assays are needed to guide clinical decision making for individual patients. In this Series paper, we discuss alignment of new tuberculosis regimens and tuberculosis DST, and we outline the actions needed for the optimum, coordinated introduction of new technologies for tuberculosis control.

Tuberculosis regimens: past, present, and future

First-line tuberculosis treatment has gradually evolved from monotherapy with streptomycin, to multidrug regimens of up to 24 months or more, and finally to the so-called short-course regimen now used in most high-burden countries.[6] This regimen is a 6 month course of treatment denoted as 2HRZE/4HR: a 2 month intensive phase of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) followed by a 4 month continuation phase of isoniazid and rifampicin. It has been the global standard first-line tuberculosis treatment for decades.

The duration of the 6 month regimen puts substantial demands on health-care systems and patients.[7,8] Meanwhile, second-line tuberculosis treatment, for patients with multidrug-resistant (MDR) tuberculosis (defined by resistance to both isoniazid and rifampicin), is based only on observational studies and expert opinion.[9] These multidrug regimens of 18–24 months are toxic, expensive, and of limited effectiveness.[10] The inadequacy of these regimens, which has become increasingly evident as more people are diagnosed with MDR tuberculosis, has led to efforts to find and develop new tuberculosis drug regimens that would shorten first-line treatment, avoid drug–drug interactions with antiretroviral therapy, and improve second-line treatment.[3,11]

Two phase 3 trials of shorter duration first-line tuberculosis treatment have now completed patient enrolment and treatment. The OFLOTUB trial[12] replaced ethambutol with the fluoroquinolone gatifloxacin in a 4 month regimen, although gatifloxacin has subsequently lost regulatory approval in many countries because of adverse events. The REMoxTB trial[13] replaced either isoniazid or ethambutol with the fluoroquinolone moxifloxacin (M) in two experimental, 4 month regimens (2HRZM/2HRM and 2MRZE/2MR). Results from REMoxTB are expected in late 2013; if positive, regulatory approval will be sought in 2014 and a national launch could start as early as 2015.

Next-generation, first-line regimens are likely to include several new drugs.[14] Clinically, the most advanced regimen[15,16] in this category is known as PaMZ, a combination of the novel nitroimidazo-oxazine PA-824, moxifloxacin, and pyrazinamide. This regimen has the potential not only to shorten the duration of first-line treatment, but also to treat a proportion of patients who would previously have needed second-line treatment—ie, patients with MDR tuberculosis.[17]

Finally, several tuberculosis drug candidates are in clinical development, but their optimised regimens have not yet been defined. Sutezolid (PNU-100480), an analogue of linezolid, is in phase 2a trials. More advanced are two new drugs that have been submitted for regulatory approval for treatment of MDR tuberculosis on the basis of phase 2b data. Bacterial burden was reduced more quickly when either bedaquiline (a diarylquinoline formerly known as TMC207)[18] or delamanid (a nitro-dihydro-imidazooxazole formerly known as OPC-67683)[19] was added, for 6 months, to an optimised background regimen for MDR tuberculosis.[18,19] Bedaquiline was granted marketing approval by the US Food and Drug Administration on Dec 28, 2012. However, the extent to which these drugs can shorten and simplify MDR tuberculosis treatment will only be known after additional, multiyear phase 3 trials.

Tuberculosis diagnostics and DST: past and present practice

For decades, tuberculosis diagnosis in high-burden countries has relied almost entirely on smear microscopy, which is inexpensive but detects only half of all cases.[10] Additionally, smear microscopy does not provide any information about drug resistance, so most patients are put directly onto a standardised first-line regimen without any knowledge of drug susceptibility. However, the increasing awareness of MDR tuberculosis[20] has drawn greater attention to the need for DST, with the initial focus on rifampicin DST for the diagnosis of MDR tuberculosis.

DST results are more likely to reach patients in a timely fashion when the DST technology allows for

implementation in simpler, more peripheral health-care settings that are closer to patients (table). The simplest health-care technologies might be suitable for the most peripheral settings (community level and health posts), but more complex technologies will be appropriate only for higher-level facilities—ie, health centres, subdistrict facilities, and larger district, provincial, and regional hospitals. The most technically demanding methods might be feasible only at the most centralised, national-level facilities (one or more of which typically operate as a reference laboratory for quality assurance).

New DST assays have been moving down this continuum; early assays were suitable only for centralised sites, but newer technologies are able to be used at more intermediate or peripheral sites. Development and field testing have led WHO to recommend automated liquid culture systems (in 2007), line-probe assays (in 2008), and the Xpert MTB/RIF test (in 2010). These systems offer benefits such as reduced time to detection of resistance (from effectively 106 days with conventional DST to 20 days with line-probe assay and less than 1 day with the Xpert MTB/RIF assay),[21] thus allowing for more rapid initiation of MDR tuberculosis treatment.[22–24] Liquid culture and line-probe assays can be implemented in national and regional reference laboratories, and the Xpert MTB/RIF assay (an automated, cartridge-based, real-time PCR assay) in more peripheral sites such as subdistrict laboratories.

Before more recent developments, the primary method for tuberculosis DST involved the culturing of *M tuberculosis*; these phenotypic growth assays are slow and need sophisticated facilities with high biocontainment. For some MDR tuberculosis drugs, even phenotypic DST is not well established, and will need to be further researched because data are insufficient to calculate clinically relevant threshold concentrations.[25] Other phenotypic (growth-based) diagnostics, such as the microscopic observation drug-susceptibility assay and the nitrate reductase assay, might be an interim solution for resource-limited settings.[26] However, due to the very slow growth of *M tuberculosis* in phenotypic assays, truly rapid testing needs a molecular approach that avoids the need to grow *M tuberculosis* and instead uses molecular biology methods to detect resistance-associated mutations in DNA. Such molecular assays are the primary focus of this Series paper.

Line-probe assays, though molecular, also present challenges. As with liquid culture, they need laboratory infrastructure that is not available at the periphery of the health-care system (eg, at health centres, district hospitals, or even most provincial hospitals), so they are not practical for routine testing of all individuals with confirmed or suspected tuberculosis in most high-burden countries.[27] Such a step would need a massive sputum sample referral and transport system that typically does not exist. Instead, cultures and line-probe assays are used largely for patients at high risk of resistance—eg, those with persistent symptoms.

The Xpert MTB/RIF test, however, has great potential because it can be used at the district or subdistrict level.[28] It not only detects rifampicin resistance, but also detects far more tuberculosis cases than does smear microscopy, particularly in regions where many people are co-infected with HIV and tuberculosis.[21] As a result, the Xpert MTB/RIF assay has been scaled up rapidly in South Africa, where it is used as the first diagnostic for all individuals

For more on the **Xpert MTB/RIF assay** see **Series** *Lancet Infect Dis* 2013; published online March 24. http://dx.doi.org/10.1016/S1473-3099(13)70008-2/

	DST in centralised laboratories (status quo for most high-burden countries)	DST in peripheral settings (eg, microscopy centres or district laboratories)
Technology requirements	Advantage: centralised laboratories allow for deployment of high-throughput, sophisticated assays (eg, microarrays, DNA sequencing, beacons, real-time PCR); these methods might be better suited to assaying many mutations and more drugs	Disadvantage: this setting might constrain technology to simpler platforms, which might not be ideal for new drugs or the addition of more drugs or mutations; the accompanying sample preparation technique should not need a laboratory with high levels of biocontainment
Cost	Advantage: centralised DST can be used only for subpopulations of patients, reducing volume and costs; samples can be batched to further increase cost efficiency	Disadvantage: DST assays for peripheral settings might be more expensive and not cost efficient (lower test volume); the overall cost of tuberculosis diagnosis might increase and health systems could be unwilling to make such big investments, unless MDR tuberculosis prevalence is very high
Quality	Advantage: quality testing and reliable results are easier to ensure in a small number of centralised laboratories	Disadvantage: unless very simple or automated, DST in the periphery will need extensive quality assurance, training, and personnel
Timeliness and use of results	Disadvantage: turnaround times are too long and losses to follow-up are high, both with samples sent and patients who never come back for results; DST results are often not reviewed when they become available, and many results never get reported or used	Advantage: if universal DST is needed at the time of tuberculosis diagnosis, then it has to be done in peripheral settings where most tuberculosis cases are diagnosed; rapid turnaround and lower losses to follow-up will mean doctors can actually act on the DST results and modify treatment decisions; they are likely to pick up MDR tuberculosis much earlier, before substantial transmission occurs
Sample transport and reporting system	Disadvantage: needs good sample transport and a reporting system, which is not available in many settings	Advantage: does not need an extensive sample transport and reporting system

DST=drug susceptibility testing. MDR=multidrug-resistant.

Table: **Advantages and disadvantages of centralised and peripheral DST**

with suspected tuberculosis. In other countries, such as Kenya, it is used for all HIV-infected individuals with suspected tuberculosis. Other resource-limited countries, however, still struggle with the cost,[29] electricity, and maintenance requirements of this assay.[30] Although the price of the Xpert technology has been reduced to under US$10 per cartridge, this negotiated price is not available to the large number[5] of patients with tuberculosis in the private health sector in some high-burden countries.[31]

The roll-out of the Xpert MTB/RIF assay has been associated with difficulties that will probably also be applicable to DST development for new tuberculosis regimens. One major issue is positive predictive value.[32,33] Even with a pooled sensitivity for rifampicin resistance of 94% and a pooled specificity of 98%,[34] the latest iteration of the Xpert MTB/RIF assay has a positive predictive value for MDR tuberculosis of only about 50% or 67% when rifampicin resistance prevalence is 1% or 2%, respectively.[32] Such resistance values are typical in new patients with tuberculosis, and the low positive predictive value results in many false positives and a substantial demand for confirmatory DST.[35] (Of note, however, even smear culture is not 100% accurate, so the true specificity of the Xpert assay for rifampicin resistance might be higher than the initially reported 98%.) In many countries with low HIV or MDR tuberculosis prevalence, the issues of positive predictive value and costs have restricted the uptake of the Xpert MTB/RIF assay.

Future needs: alignment of new drug regimens and new diagnostics

Selecting drugs to test and ways to test them

Which of the new drugs are the most important targets for future DST? Typically, DST has focused on drugs for which resistance has one or more of three consequences: it undermines treatment effectiveness, it increases the risk of resistance amplification, or it strongly predicts resistance to other drugs (ie, acts as a triage assay). At present, rifampicin DST has been prioritised to diagnose MDR tuberculosis.[36] Evidence suggests isoniazid DST should also be done: substantial numbers of patients harbour isoniazid-resistant, rifampicin-susceptible strains, and patients with such strains have reduced treatment success.[37,38] For implementation of the 4 month regimens, DST to detect susceptibility to rifampicin and fluoroquinolones will be of interest, especially in countries that already do DST for rifampicin. For the PaMZ regimen, a rapid test for moxifloxacin and pyrazinamide would probably be the first priority, because clinically significant resistance to PA-824 has not yet been shown. Development of DST for PA-824 and other new drugs will be prioritised—initially for use in surveillance—as resistance to them develops and their use becomes more widespread.

After deciding which drugs to test, additional information is needed. To be rapid and clinically useful, a DST assay will probably need to be molecular. Therefore, information about resistance mutations—and the correlation of those mutations with clinical outcomes—is needed to form the basis for such a test.

The Xpert MTB/RIF assay's 94% sensitivity for detection of rifampicin resistance is only possible because almost every mutation contributing to rifampicin resistance is known and present in a short, defined DNA region. For fluoroquinolones, however, incomplete knowledge of all contributing resistance mutations outside the quinolone-resistance determining regions of *gyrA* and *gyrB* means that sensitivity with such molecular methods would, on the basis of current knowledge, be limited to about 85%.[26,39] As occurred recently for a line-probe assay for second-line drugs, when an assay has insufficient sensitivity, it might be recommended for use as a rule-in test only.[10,35] Sensitivity might be enhanced by incorporation of additional, low-abundance mutations, but doing so might reduce specificity to an unacceptable level—eg, if specificity for each of five independent mutations is 98%, the overall specificity of a test including all five mutations would be $0{\cdot}98^5$ or 90%. Other major issues, for fluoroquinolones and other drugs, are the possibility of multigenic resistance and the difficulty of detecting already-known mutations from a mixed population of bacilli.[40]

DST for pyrazinamide poses even more challenges. The activation of pyrazinamide requires pH levels that are difficult to maintain in culture media, so phenotypic DST for pyrazinamide is inconsistent. Analysis of the sequence of one resistance gene (*pncA*) has been proposed as an alternative, although this approach might detect only about 90% of pyrazinamide resistance.[41] The mutations are spread along the entire length of the *pncA* gene, however, necessitating analysis of a fragment of roughly 700 bp. This drawback has led to the idea of testing for the presence of a wild-type gene (rather than testing for the presence of a specific mutation) as a way of ruling out resistance. In this approach, silent mutations, which do not confer resistance, would probably prevent hybridisation and thus yield false positives. These silent mutations, although rare,[42] need to be better characterised by standardised and validated culture-based pyrazinamide resistance assays and incorporated into a molecular testing algorithm.

To minimise these limitations, one priority in translational science is to link gene mutations to phenotypic resistance (ie, the amount of drug needed to inhibit bacterial growth).[43,44] A second priority is to develop strain collections (preferably sequenced[45,46]) that will assist with the testing of new diagnostic assays and the development of genomic databases that would predict drug susceptibility phenotypes. For new drugs, isolates that develop resistance in vitro should be stored for later assessment, but their clinical significance will be unclear until resistance is noted in clinical use. Compound availability for such clinical assessment and data for

crucial breakpoints are likely to emerge only after regulatory approval of new tuberculosis drugs. Post-marketing studies will be important to identify treatment failures and resistance mechanisms.

Surveillance: a basis for decision making

Once translational science has provided a means to detect resistance, the next task will be to establish existing or emerging resistance levels via surveillance. Data for global drug resistance obtained through WHO's Global Project on Anti-TB Drug Resistance Surveillance is available from 135 of 194 member states, of which only 63 countries have continuous surveillance systems that use DST.[10] Generally, surveillance is restricted to activities that align with current rather than future treatment priorities. Most countries assess resistance to isoniazid, rifampicin, and ethambutol (pyrazinamide is often excluded, because of the methodological challenges already discussed) in new and retreated patients. Resistance to fluoroquinolones is assessed only in patients with MDR tuberculosis because these patients are the only ones recommended to take fluoroquinolones by WHO and the International Union Against Tuberculosis and Lung Diseases; however, a substantial amount of fluoroquinolone use is believed to occur in first-line tuberculosis treatment in the private sector of some countries.[5]

Such data are insufficient to assess development and implementation priorities for new tuberculosis regimens and diagnostics. The key information gap for the REMoxTB regimens is fluoroquinolone resistance in new patients. Although existing data suggest that such resistance is very low in most[47–49] but not all[50,51] countries, the absence of such data for most high-burden countries makes it difficult for a country to assess the cost-effectiveness of the new regimen (ie, one factor in deciding whether to implement) or the most appropriate DST algorithm (ie, how to implement). And, for PaMZ, pyrazinamide resistance rates in both new and MDR tuberculosis patients are missing. For both moxifloxacin and pyrazinamide resistance, some data are available from clinical trials, but nationally representative data are sorely needed. As new drugs with new mechanisms of action are adopted, surveillance will also be needed to monitor for the development of resistance to bedaquiline, delamanid, and others.

For surveillance data to be meaningful, the data should be representative of either a national or subnational population, be obtained using quality-assured assays, and distinguish between resistance rates in new patients and retreated patients. Ideally, DST surveys should be linked with treatment outcomes and patient care (although methods with high quality assurance would be needed) and would make use of new, high-throughput molecular methods that would be much faster than current growth-based assays. For example, with a sufficient foundation of mutation data,[43] sequence-based assays can provide rapid and accurate information and, for many drugs, good correlation with DST obtained with liquid culture.[46]

DNA sequencing—as a centralised procedure—is more practical for surveillance than for patient care. But even for surveillance, it is important to develop fast and safe specimen preparation, transport methods that maintain stability of the DNA in the specimen, and templates, primers, barcodes, and standardised electronic reporting. Such systems should improve in accuracy as mutations with unknown association are obtained and analysed; however, while this knowledge is being accumulated, parallel implementation of phenotypic and molecular assays might be needed.

Collaboration with a country undertaking a drug resistance survey could provide an opportunity to pilot the technology and develop the systems described above. Such a study could provide the proof of principle and the data to validate such a system.

Modelling of alternative DST strategies

Drugs and diagnostics are implemented as individual elements of a larger, more complex tuberculosis management system. In the public health approach, all incoming patients are subdivided into just a few treatment pathways. Central to this management system are diagnostic algorithms, which consist of different permutations of drugs to test for, the level of the health-care system at which the testing is done, the selection of the patient population eligible for testing, and decisions about single-step or multiple-step testing. At the end is a treatment decision. New regimens introduce several new variables to consider when deciding which algorithms are most effective, and data to inform this decision will be scarce at the time any new regimen is introduced. Mathematical models can be useful to guide decision making in such instances in which direct data are scarce.[52]

Such models use existing data to simulate simplified tuberculosis epidemics that behave according to best current knowledge. These models can then be used to project the medium-term incidence and prevalence of drug-resistant tuberculosis at the population level under various assumptions about the deployment of new regimens and corresponding DST.

For example, one priority question is where DST should be placed in treatment algorithms for various epidemiological and economic contexts. Clearly, the ideal algorithm (from a perspective of reducing drug resistance) is to deploy DST for all people with confirmed or suspected tuberculosis, with confirmatory testing of preliminary positives. Preliminary modelling has suggested that the so-called test-early strategy for isoniazid and rifampicin might be cost effective in areas with an underlying MDR tuberculosis prevalence as low as 2·1%.[53] However, this strategy is only feasible in areas where good DST exists for a given regimen, resources are sufficient to deploy such DST widely, and use of DST will not greatly delay initiation

of treatment. Most high-burden settings therefore cannot consider such algorithms at this time.

A history of previous treatment is a strong independent risk factor for resistance, so DST should be directed at these subpopulations. But when should DST be implemented more broadly? For large public health programmes in resource-limited high-burden countries, it might make sense to implement DST only when the prevalence of resistance to a given drug rises above a specific threshold. Below this level, the implementation challenges and issue of false positives outweigh the risks from undetected resistance. Above this level, action is needed to prevent worsening treatment outcomes, resistance amplification, and increased transmission. But generally the point at which this threshold should be set in different epidemiological and economic conditions is unclear—especially when MDR tuberculosis hot spots occur within countries that otherwise have low overall prevalence.[54] The answer will also vary depending on whether the remaining drugs in the regimen will still protect the person from resistance generation and disease progression. Modelling could help to assess which thresholds make sense in terms of public health benefit, cost, and cost-effectiveness.

Two questions have arisen in discussions of modelling DST in the context of new tuberculosis regimens. First, what would different DST assays—with different speed, accuracy, price, and technical specifications (ie, which drugs, how many mutations)—achieve in terms of a population-level effect and cost-effectiveness, and what are the trade-offs between these various specifications? Second, what is the population-level effect and cost-effectiveness of different DST algorithms (eg, DST for all, DST for only patients who are being re-treated or in whom previous treatment had failed, or use of new regimens without DST) as a function of baseline drug resistance and rate of emerging resistance?

Those deciding how to deploy DST should consider the projected epidemiological outcomes, budgetary constraints, feasibility concerns, and political realities. Mathematical models can assist with the first of these (projections of potential outcomes), and thereby serve as an important tool for decision makers. However, these models are restricted by the quality of data; in particular, data are sparse for the extent of drug resistance in many high-burden areas and the rate at which resistance to second-line drugs (eg, fluoroquinolones) might emerge under pressure from new regimens. Thus, even when restricted to the outcomes issue, mathematical models cannot validate which assumptions about emergence of drug resistance are correct. However, they can project epidemiological outcomes under best-guess assumptions of these data-points, describe the range of uncertainty, emphasise the data for which surveillance is most crucial as new regimens are deployed, and provide preliminary guidance in line with current knowledge while those data are obtained.

Development of new DST assays

Information about resistance rates (from surveillance) and algorithm choice (from modelling) can directly inform the final question: what new DST assays need to be developed? A target product profile (TPP) is a list of product specifications, including projected product performance and target patient population. The TPP of a DST assay will vary depending on the intended use (individual treatment decisions *vs* surveillance), the epidemiology (detecting low *vs* high resistance), the health-system context (where it is positioned in possible algorithms), and whether the technology will be used in central or peripheral settings.

Example TPPs and DST approaches have been described elsewhere.[55,56] Beyond the target drug(s), these TPPs should address several issues: what is meant by rapid; what level of sensitivity and specificity a DST assay needs for it to be practical and feasible; what other diseases should be able to use the same DST platform technology; and what level of complexity, containment, and cost are needed.

But two related issues stand out. First, should DST be bundled into case-detection assays (as with the Xpert MTB/RIF assay), or should it be a reflex test that is done only after tuberculosis is diagnosed? Of the two approaches, reflex testing needs more patient samples (and potentially more patient visits, with associated loss to follow-up and delays in treatment initiation). But reflex testing means that only patients with confirmed, rather than suspected, tuberculosis undergo DST, which can greatly reduce costs.

Second, new DST assays could be developed for deployment at either centralised laboratories or the more

Panel 1: Diagnostics developers' requirements beyond target product profiles[63]

Potential market size

- Size of the target population
- Market reach of competing drug susceptibility testing technologies
- Diagnostic algorithms used now and in the future; current and future tuberculosis treatment landscape
- Segmentation of markets by income and by peripheral versus centralised methods
- Projected scale-up dynamics

Practical steps

- Sources of funding and technical support, especially for validation trials
- Whether validation trials can address only accuracy or also have to show clinical effect
- Requirements for regulatory and policy approvals
- Potential procurement and scale-up challenges at the country level

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peripheral levels of the health-care system (table). Emerging technologies for DST are abundant—including microarrays, next-generation sequencing, line-probe assays, molecular beacons, high-resolution melt curve analysis, lights on/lights off technology, cyclic catcher melt temperature analysis, phenotypic colour tests, pyrazinamidase assays, and combination phage and molecular assays—and some of these technologies can be readily adapted to increase the number of mutations detected, but few are suited to use in peripheral laboratories.

Therefore, investment will be needed either to develop cost-effective and robust DST methods for peripheral laboratories, or to create rapid, reliable sample transport systems to support centralised DST (along with mobile-health and patient-incentive solutions to reduce delays and dropouts). Deployment of testing at the point of treatment can bring obvious advantages, such as reduced delay and dropout, but can add substantially to the overall cost because of the many instruments needed and the lower volumes of testing per site.[57]

Many countries diagnose drug-sensitive tuberculosis at the peripheral levels of the health system, but initiate treatment at the subdistrict level. Therefore, a compromise might be to have a new, sensitive case-detection assay as a true point-of-care assay, followed by DST given as a reflex assay at subdistrict level at the time of treatment initiation.

If non-centralised DST remains the strategy, simplicity should be a major goal.[58] Simplified smear microscopy algorithms provide an interesting example of how up-front performance (in this case, sensitivity) is sometimes worth sacrificing in return for a protocol that is simpler for the patient (with lower travel costs) and that therefore is associated with less dropout and better overall effectiveness.[59,60] Modelling studies[61,62] have already resulted in similar conclusions for new diagnostics. Improved assay sensitivity provides some epidemiological gains, but the greater population effect comes from a focus on test specifications that allow peripheral use and fast turnaround times, thus reducing patient delays and default.[61,62]

One option for a peripheral laboratory test is to focus on excluding all patients who are likely to be resistant; high sensitivity becomes the goal and specificity becomes less important. A test with lower specificity can be acceptable if the prevalence of resistance is high, if an effective and safe alternative regimen (eg, 2HRZE/4RH for PaMZ) is available, or if used as a triage test. One example of an approach that prioritises sensitivity is the proposed molecular assay to screen for the wild-type *pncA* gene as a correlate for pyrazinamide susceptibility, rather than trying to capture the many different *pncA* mutations that can lead to pyrazinamide resistance. Another option is to continue—even with new regimens—to focus on rifampicin resistance screening as a first step. Preliminary evidence[17] suggests that rifampicin-resistant strains are more likely than rifampicin-sensitive strains to be resistant to pyrazinamide and fluoroquinolones. Therefore, DST for rifampicin might be a useful triage test even if the first-line regimen does not contain rifampicin (eg, PaMZ). The subsequent pyrazinamide and fluoroquinolone DST could then be restricted to a smaller population with a higher prevalence of resistance.

All of this theory is irrelevant unless companies invest in the development and testing of new tuberculosis diagnostics. These developers should be aware of what is needed in resource-limited settings and be willing to take a product all the way through field testing to

Panel 2: **Framework to achieve successful implementation of new tuberculosis regimens and drug susceptibility testing (DST)**

Short term

- Identify all mutations in *Mycobacterium tuberculosis* that occur reasonably frequently and that result in resistance to existing and new drugs; priority should be placed on obtaining resistance information from clinical samples that are accompanied by treatment outcome data
- Develop a collection of sequenced sensitive and resistant strains that can be used to assess new DST assays
- Use modelling to define which strategies for deployment of DST will have the greatest population-level effect and be most cost effective; various strategies would include different DST assays that vary in their speed, sensitivity and specificity, cost, and technical specifications and different DST algorithms, used in the context of various baseline resistance levels
- Undertake surveillance of moxifloxacin resistance in new patients with tuberculosis and of pyrazinamide resistance in new and previously treated patients, and patients with and without multidrug-resistant tuberculosis
- Do operational research to assess and optimise systems for sputum transport and reporting results (including prompt initiation of treatment in response)
- Develop clear target product profiles to guide diagnostics developers about the necessary product specifications and likely market demand
- Do analyses of the tuberculosis diagnostics market size and potential to inform investment decisions by test developers

Medium term

- Use existing diagnostics platforms to develop, field test, and commercialise DST assays—particularly for fluoroquinolones and pyrazinamide—that can be implemented at the subdistrict level
- Monitor for clinical resistance generated during the roll-out of new tuberculosis drugs (ie, new chemical entities) and identify the molecular basis for such resistance
- Refine models of long-term impact based on early surveillance data during roll-out of novel regimens
- Develop DST assays for new tuberculosis drugs and use them to do ongoing surveillance
- Develop and strengthen systems for using next-generation sequencing for tuberculosis drug surveillance

Long term

- Develop new diagnostic platforms that are rapid, inexpensive, and can be implemented at the subdistrict level
- Develop a universal regimen for tuberculosis that has at least three novel chemical entities and that therefore minimises the need for DST while treating all forms of tuberculosis

Search strategy and selection criteria

This Series paper draws on material from a meeting of the Tuberculosis Diagnostics Research Forum sponsored by the Bill & Melinda Gates Foundation and the US National Institutes of Health held on Oct 1–2, 2012, in Arlington, VA, USA. Additionally, we identified references for this review by searching PubMed with a focus on articles published between January, 2008, and November, 2012. Search terms included, but were not restricted to, "tuberculosis", "drug susceptibility testing", "drugs", "diagnostics", "drug resistance", "surveillance", and "point-of-care testing". We did not apply language restrictions. Additional information came from our personal collections of peer-reviewed papers, from the reference lists of identified papers, and from reviewers.

commercialisation. The perception that these assays have little commercial opportunity is a substantial barrier to development, and supportive financing will probably still be needed. In addition to the TPP issues listed previously, diagnostics developers are interested in potential market size and the practical steps needed for test development, validation, regulation, and policy (panel 1).[63]

Developers targeting surveillance have a particularly small market, although the barrier to entry is much lower because these high-throughput, centralised machines can be built on the presumption that users will have a high level of skill and that the machine will have applications beyond tuberculosis. For developers interested in peripheral DST for patient care, the demands in terms of assay simplification and robustness increase greatly, and market size is very dependent on the resistance thresholds for testing. Test developers might therefore be more interested in a product that combines tuberculosis detection and DST because this product will have a larger market than a DST-only product.

Private sector procurement is a major strategic gap. If new DST assays are highly priced, few private practitioners will use them, and DST will be missing from the sector that is most likely to adopt new drugs quickly and in the context of variable regimens. To solve this issue, a mechanism is needed to ensure that private laboratories pass along any savings from assays purchased at concessionary prices.

Although demonstration projects for diagnostics need substantial investment, assay development with existing platforms can be cheap by comparison. But even to make these investments, diagnostic companies need a prediction of user needs (where the user is often a national tuberculosis programme) and market demand. Defining a clear set of specifications for the desired DST—and the likely demand for such DST—is the next major point of collaboration for drug and diagnostic developers.

Conclusion

The prospect of new tuberculosis regimens is exciting, because patients have had to rely on a single lengthy treatment option for decades. Several opportunities are available to mitigate the risks of developing resistance to these new regimens. Assays to detect resistance can be developed before repurposed drugs come to market and early in the implementation of new drugs. Surveillance DST can identify areas where some regimens might be compromised by high levels of background resistance, and treatment decisions can, in some settings, be tailored to the individual by rapid DST before treatment. Modelling studies will help to assess costs, outcomes, and feasibility to predict implementation approaches. Panel 2 outlines a framework to achieve these goals. When all of these strategies are brought to bear, drugs and diagnostics will together make a powerful combination.

Contributors

AZ and MS initiated this Series paper. WAW led the writing of the paper, and all authors contributed equally.

Members of the Tuberculosis Diagnostics Research Forum

The decision to submit the report for publication was made by the primary authors, who also drafted the manuscript text. However, ideas also arose from discussion at a meeting (see Search strategy and selection criteria). Attendees at this meeting included: David Alland (New Jersey Medical School, Newark, NJ, USA); Naomi Aronson (Uniformed Services University of the Health Sciences, Bethesda, MD, USA); Helena Boshoff (National Institutes of Health [NIH], Bethesda, MD, USA); Ted Cohen (Brigham and Women's Hospital/Harvard School of Public Health, Boston, MA, USA); Luke T Daum (Longhorn Vaccines and Diagnostics, San Antonio, TX, USA); Nila Dharan (University of Medicine and Dentistry of New Jersey, Newark, NJ, USA); David L Dolinger (Seegene, Gaithersburg, MD, USA); Matthias Egger (University of Bern, Bern, Switzerland); Kathleen Eisenach (University of Arkansas for Medical Sciences, Little Rock, AR, USA); Diane Flayhart (Becton Dickinson, Franklin Lakes, NJ, USA); Michael Hoelscher (University of Munich, Munich, Germany); Sven Hoffner (Swedish Institute for Communicable Disease Control, Solna, Sweden); Eric Houpt (University of Virginia, Charlottesville, VA, USA); Robin Huebner (NIH); Patrick Jean-Philippe (Henry M Jackson Foundation and NIH, Bethesda, MD, USA); Lee Pyne-Mercier (Bill & Melinda Gates Foundation, Seattle, WA, USA); Timothy Rodwell (University of California, San Diego, CA, USA); Christine Sizemore (NIH); Sudha Srinivasan (NIH); Faramarz Valafar (San Diego State University, San Diego, CA, USA); Richard White (London School of Hygiene and Tropical Medicine, London, UK), and Sharon Williams (NIH).

Conflicts of interest

WAW, EG, and KM are employed by the TB Alliance, whose mission is to develop new, improved regimens for tuberculosis. FGJC serves as a consultant to the Foundation for Innovative New Diagnostics (FIND). FGJC and MP serve as consultants for the Bill & Melinda Gates Foundation. All the other authors declare that they have no conflicts of interest.

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policies of the US Department of Health and Human Services or the authors' national governments, nor does mention of trade names, commercial practices, or organisations imply endorsement by the US Government or the authors' national governments.

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