Viewpoint TB Diagnostics: What Does the World Really Need?

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Stagnant tuberculosis (TB) case detection and rising TB drug resistance are in part the result of historically neglected laboratory services, slow technology transfer, and a lack of new TB diagnostic tools. The last decade has, however, seen the diagnostic pipeline grow rapidly, with research and investment prompted by concerns about the global spread of drug resistance and transmission in human immunodeficiency virus (HIV) settings. The drawbacks of conventional microbiological methods for TB diagnosis and resistance detection have largely been overcome by modern molecular technologies; however, the much needed point-of-care TB test will remain elusive if expectations stay unrealistic and research and funding strategies are not changed. Development of new technologies, better use of existing tools, and adequate treatment capacity to care for the increasing numbers of cases that will be diagnosed with scale-up of TB diagnostics all need to be addressed simultaneously.

After a decade of rising tuberculosis (TB) case detection globally, case notification rates are slowing down in the Americas and the European region, and have been static for the last 5 years in the Western Pacific. Although the incidence of TB is estimated to have peaked at 142 per 100 000 population in 2004, declining in 2009 to 137 per 100 000, the absolute number of cases continues to grow with the increase of the world’s population. In 2009, 9.4 million cases were estimated to have occurred, with 1.8 million deaths [1]. Figure 1 shows the highest incidence areas where access to health services and achieving a diagnosis of TB are most difficult, notably sub-Saharan Africa.

Meanwhile, increases in drug-resistant TB have been recorded globally (Figure 2), and notably the countries of the former Soviet Union have seen significant increases in multi–drug resistant forms of TB in the last 15 years [2]. The paucity of data for many areas in the world is disturbing, particularly in sub-Saharan Africa and parts of southeast Asia, as a direct consequence of grossly neglected laboratory services.

One of the major bottlenecks in TB control is the difficulty in making the diagnosis. Ever since the discovery of Mycobacterium tuberculosis by Robert Koch in 1882, the scientific world has been on a quest to develop reliable, rapid, and robust diagnostic tests. The century that followed saw many claims made—and fade—as the organism proved to be more elusive and difficult to diagnose than scientists had anticipated. Conventional microbiology seemed to be the answer and sputum smear microscopy became the mainstay of TB diagnosis shortly after Koch’s discovery, to this day remaining the most widely used diagnostic option in low-income countries.

CURRENT DIAGNOSTICS FAILING TB CARE AND CONTROL

The limitations of microscopy are well known, with low sensitivity (40%–60% when compared with culture) being the most pressing. The human immunodeficiency virus (HIV) epidemic has put additional pressure on microscopy by increasing the proportion of smear-negative cases and those with extrapulmonary disease, which together now account for as much as 60% of all TB notifications in many settings, particularly in those with high HIV prevalence [1].
The effectiveness of microscopy is further diminished by the conditions in which laboratory staff work—with poor microscopes, dubious reagents, variable electricity, insufficient time to examine slides properly, and little value placed on their work. Infectious cases therefore remain undetected and thus untreated, and must in part have contributed to the size of the epidemic seen today.

Koch’s work on the anthrax bacillus grown in pure culture paved the way for understanding the fundamental and underlying etiology of infectious diseases, including TB. Culture of *M. tuberculosis* is highly sensitive and the current gold standard for diagnosis but unfortunately suffers from many drawbacks, notably time delays. Sophisticated biosafety and infrastructure requirements, specialized laboratory staff skills, and high testing costs also have to be met. These are compounded when isolates of *M. tuberculosis* have to be manipulated to assess in vitro drug resistance, with conventional growth-based drug susceptibility testing (DST) adding further delays. In HIV settings a diagnosis of multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB often reaches the health facility after the patient has died [3]. In addition, multiple outbreaks of HIV-associated MDR-TB began in the late 1980s and are still being reported today [4].

Improvements in conventional culture and DST methods have been slow and incremental. The use of commercial liquid culture systems significantly reduced the turnaround time of results (from months to weeks) but came at a cost out of reach of most low-income countries until 2007, when WHO endorsement and price negotiations by FIND (Foundation for Innovative New Diagnostics) resulted in preferential pricing for these countries. Selected non-commercial culture and DST methods were endorsed by WHO in 2009, reducing the cost of testing but not the operational needs for sophisticated laboratory infrastructure and biosafety, or the requirement for specially trained laboratory staff [5].

As a result of the limitations of current technology, TB patients in resource-limited settings are often diagnosed late, and drug-resistant TB often remains undiagnosed and untreated. Sputum smear microscopy has been “king” for over a century and like the English monarch Charles II, is “an unconscionable time a-dying.” With case notifications static globally the question is whether we are reaching the limits of detection with sputum smear microscopy, and whether the “king” has—for the first time—a real contender for the throne.
ENTER MODERN TECHNOLOGIES

In the early 1980s, the polymerase chain reaction (PCR) became the first molecular method to amplify nucleic acid sequences, and molecular diagnostics were widely expected to have a major impact on clinical medicine. However, this early promise was unfulfilled in TB diagnosis, largely due to the complexities of DNA extraction, amplification, and detection, and the biosafety concerns related to manipulating *M. tuberculosis* organisms. In addition, commercial nucleic acid amplification tests (NAAT) proved to be significantly less sensitive than microbiological culture, especially for smear-negative TB. Moreover, culture largely remained necessary as a precursor to DST, while scale-up of conventional laboratory services remained slow and expensive, requiring major investment in infrastructure and human resources.

Line probe assay (LPA) brought the first significant breakthrough, detecting rifampicin and isoniazid resistance within 24–48 hours. Finally, a rapid, high throughput technology to detect MDR-TB was available, albeit suitable at reference laboratory level, and in smear-positive specimens only. The king was not yet dead. Endorsement of LPA by WHO in 2008 was accompanied by guidance on several operational considerations for implementation, notably the need for sophisticated laboratory infrastructure and human resource skills [6]. And rapid diagnosis of TB with molecular tools was not yet a reality.

Enter the GeneXpert platform, launched in 2004 to provide simplified molecular detection of anthrax bacilli by integrating and automating sample preparation, amplification, and detection through PCR-based technology. Using a 6-color laser detection device, diagnosis was obtained within 100 minutes. The Xpert MTB/RIF [7] cartridge, for the simultaneous detection of TB and rifampicin resistance, took 4 years to develop, followed by the process of attracting funding to refine and manufacture the technology and then conducting the clinical trials that established its effectiveness.

A unique collaboration between academia and industry, brokered by FIND, proved to be the key ingredient in bringing this new technology to market in record time and enabling WHO to assess the evidence base and rapidly develop policy for implementation. The innovative approach followed in Xpert MTB/RIF development and evaluation stands out as a pathfinder: the needs of users in the field dictated product specifications; adequate research funding was provided by the US National Institutes of Health and the Bill and Melinda Gates Foundation; academic partners collaborated on the core
components of the technology, laboratory validation was facilitated by resources such as the FIND specimen bank, an expression library representing all described rpoB mutations that enabled validation of the rpoB assay, and libraries of other pathogens (notable nontuberculous mycobacteria) that helped confirm test specificity; large-scale field evaluations were carried out by FIND under well-designed operational research protocols; and industry was responsive and flexible in engaging early on with FIND in contract negotiations on cost and preferential pricing. The product was a fully automated, closed (and therefore safe) real-time PCR system, requiring basic worker skills and no specialized laboratory infrastructure.

Is Xpert MTB/RIF the ultimate point of care (POC) test? Unfortunately not, although the performance characteristics of the system meet—and even exceed—most of the recently suggested requirements for a POC test [8, 9]. The development of Xpert MTB/RIF has, however, set the stage for moving from widely outdated to 21st century TB diagnostics. Finally, the king may be dying, and the question therefore is, what must the prince (or princess)-in-waiting look like? We contend that a reality check is needed to distinguish between a wish list for the perfect POC test and the reality of what can be achieved given a complex organism, the current pace of technology development, and clinically complex treatment requirements.

**POINT OF CARE DIAGNOSTICS—RESEARCH TOWARD AN IMPOSSIBLE DREAM?**

Current convention holds that maximum clinical and public health impact of new diagnostics for TB will require a POC test widely accessible at the patient level. Characteristics of an ideal test include high accuracy (sensitivity and specificity), speed (a few hours at most), robustness (non–instrument-dependent, temperature- and humidity-tolerant), ease of use (noninvasive specimens, usable by nonskilled individuals), safety, and affordability [9].

Ideally, a POC test should also screen for drug resistance and positively impact clinical decision-making, reduce health care staff workload, improve patient outcomes, and change health economics. A tall order, with the challenges manifold: advancing already identified biomarkers in POC diagnostic format or identifying new biomarkers specifically for POC tests, and developing a simple and low-cost POC test for use in resource-constrained developing countries.

**Advancing Already Identified Biomarkers in POC Diagnostic Format**

POC tests usually have lower sensitivity and specificity than laboratory-based equivalents, and tests aimed at measuring host response have been most disappointing. Despite being widely marketed as a rapid and accurate technology for TB diagnosis, commercial serodiagnostic tests have a dismal performance record, to the extent that WHO has recently issued policy guidance against their use [10]. Research efforts toward antibody or antigen detection tests therefore need major overhaul and innovation.

Nucleic acid amplification testing is arguably the most appealing current option for TB diagnostic development. DNA-based tests targeting the rpoB gene are most advanced, given the specific sequence for *M. tuberculosis* contained in the rpoB gene and the number of well-characterized mutations that confer resistance to rifampicin (as a reliable surrogate for MDR) within a small region of 81bp. However, due to the technical complexity of DNA extraction, amplification, and detection, NAAT technology is not easily translated into a POC device, as demonstrated by the lack of a successful POC NAAT for any disease.

**Identifying New Biomarkers Specifically for POC Tests**

Biomarker discovery research in TB has traditionally been directed at finding reliable surrogates for culture to assess/predict treatment prognosis and has only recently become a focus for diagnostic development. The era of “omics” has seen large-scale searches for biological markers of disease and application of emerging technologies to identify novel markers of disease, particularly from blood and urine. Techniques including proteomics, transcriptomics, lipomics, and metabolomics have, however, so far failed to result in a viable test product.

**Developing a Low-Cost POC Test for Use in Resource-Constrained Developing Countries**

A major barrier to TB diagnostic test development is a lack of cohesion and collaboration between funders, academic partners, industry, and end-users. Current funding streams are not conducive to efficient discovery and do not take advantage of the screening methods that can be harnessed by industry. Researchers publish only limited information given the competition for research funding and potential financial benefits related to patenting. Tests are most often developed in low TB and HIV prevalence settings, with results not applicable to high-burden settings. Further validation under different epidemiological conditions is critical yet often beyond the financial and logistical limits of small biotech companies and academic research units. Moreover, evaluation studies often suffer from critical gaps in study design and scientific rigor, precluding sensible interpretation of results.

In short, if the challenges of POC test development for TB are to be overcome, increased collaboration and openness between researchers, academic institutions, and industry must start now, supported by adequate funding that also extends to large-scale field evaluation studies. The Xpert MTB/RIF collaboration mentioned above may serve as an example of successfully bringing together industrial and academic groups to develop appropriate tests. Such a collaborative model would need (1) frameworks for intellectual property sharing, (2) clear product specifications that are technologically feasible, (3) adequate funding with clear milestones and indicators for achievement, and (4) a funding model that incentivizes industry involvement.
It is our contention that without a significant change in funding and research strategies, the primary goals of a useful POC for TB diagnosis are years away. Low initial cost should not be the primary criterion for a new diagnostic test. Much more important would be to focus on innovative pricing structures for the developing world. With a useful product, demand, community advocacy, and political commitment will drive up sales. The resulting increased volumes will enable competition, and price is likely to fall. Likewise, negotiations with suppliers involving innovative mechanisms such as pooled procurement also drives down prices, similar to what has been seen with antiretroviral therapy and to some extent with second-line drugs for MDR-TB.

MORE THAN JUST TECHNOLOGIES NEEDED

Prior to Xpert MTB/RIF, available technologies for TB diagnosis and DST all suffered from one major drawback: the need for sophisticated and expensive laboratory infrastructure and highly skilled laboratory technicians. These needs can be reduced by wide-scale introduction of Xpert MTB/RIF; however, they are not completely eliminated, as every country still requires adequate capacity to monitor TB and MDR-TB treatment by conventional methods and to conduct DST for drugs other than rifampicin.

Establishing and improving conventional culture and DST capacity is expensive and time consuming, requiring major overhaul of TB laboratory services. Funding by UNITAID and other donors to the 27 recipient countries in the EXPAND-TB project (a joint project by WHO, the Global Laboratory Initiative (GLI), FIND, and the Global Drug Facility (GDF) aimed at expanding and accelerating access to new diagnostics for MDR-TB) has kick-started this process and has shown the benefit of a coordinated approach to ensure that implementation of new technologies is accompanied by the other essential components of laboratory strengthening (policy reform, infrastructure development, commodity and supply chain management, training and quality assurance, and monitoring and evaluation). Key features of the EXPAND-TB project include:

Ownership by Ministries of Health and National TB Programs, culminating in rapid technology transfer and policy reform at country level;

Long-term, on-site mentoring and capacity development, ensuring sustainability of local skills and competencies in a “learning-by-doing” approach;

Flexible and collaborative partnerships, ensuring optimal use of resources and avoiding duplication and overlap of laboratory strengthening activities at country level.

Introduction of Xpert MTB/RIF and future new technologies would require similar implementation models, given that TB diagnostics—at least for the foreseeable future—would have to be used in combination in country-specific diagnostic algorithms and in tiered laboratory services.

MORE EFFECTIVE USE OF SCREENING TOOLS TO OPTIMIZE NEW DIAGNOSTIC TECHNOLOGIES

The primary aim in TB diagnostic development is to confirm TB using so-called rule-in tests. Until now, very little consideration has been given to so-called rule-out tests that, while they will never be a complete solution, could be delivered within a foreseeable timeframe. Such tests would have value by reducing the number of patients requiring more complex investigations or referral, and are likely to be used in conjunction with algorithms (based on risk factors and/or symptoms and signs) that can easily be applied by relatively unskilled health care workers. Currently, there is very little research exploring the role of clinical and other algorithms to rule out TB disease; however, Xpert MTB/RIF implementation has prompted another look at the value of chest X-rays in addition to clinical screening.

Radiology started only a few years after Koch, with Roentgen’s discovery of X-rays, but never became even a “prince” in TB control. Over the past decades, public health policy discouraged the use of chest radiography as a diagnostic tool for TB since it is nonspecific and reliance on it alone as a diagnostic test results in considerable overdiagnosis [11]. Further major problems include the often-poor quality of chest X-ray films, particularly in low-resource settings, as well as the unreliability of their interpretation.

Widespread introduction of digital radiology may address the challenge of poor quality, while concordance of X-ray interpretation may be improved through standardized reading and recording [12]. Computer-aided diagnosis (CAD) has been helpful in interpreting medical images, with studies from other lung diseases suggesting that CAD may detect half of the lesions overlooked by human readers of chest radiography with a minimal number of false positives [13].

While CAD and digital chest X-rays show promise, not enough has been done to explore their role in TB screening. We suggest that this area, too, would benefit from the kind of collaboration between policy makers, end-users, and industrial and academic groups that has been successful in bringing the Xpert MTB/RIF assay to the point of introduction into National TB Programs.

IMPROVED DIAGNOSIS: ARE WE READY WITH TREATMENT AND CARE DELIVERY?

An imminent expansion in diagnostic capacity of MDR-TB in low-income countries begs the question whether treatment services are ready to cope with the influx of additional patients detected.
The provision of care for MDR-TB cases in such countries has been guided over the past decade by the Green Light Committee (GLC) Initiative [14], a partnership of WHO, high MDR-TB burden countries, development agencies, and nongovernmental organizations, which has supported countries to develop MDR-TB treatment projects. Since its inception, the GLC has approved more than 105,000 patients for treatment, of which only about 35,000 have started [15]. Recognizing that these numbers are only a tiny proportion of the 440,000 cases of MDR-TB estimated to occur annually [16], the GLC has undergone restructuring and decentralization and is now focusing on scaled-up, nationwide provision of care for patients with MDR-TB. However, significant obstacles remain to wider treatment, most importantly the apparent reluctance of governments to invest in treating MDR-TB given its greater complexity, difficulty, and costs.

Readiness at national level to treat all those that new technologies could diagnose first requires overcoming this reluctance. Resolution 62.15 at the World Health Assembly 2009, committing all countries to expanding provision of care for MDR-TB [17], was an important first step; however, much more is required, including policies for how, where, and by whom patients with MDR-TB will be diagnosed and treated. Regulations ensuring the quality, registration, and importation of second-line drugs will need revision in many countries [18]. In those few that manufacture such drugs, quality assurance to international standards is necessary. Sufficient hospital beds need to be found to admit those needing treatment, or policies need to be developed and disseminated for community management of cases. Sufficient staff will need to be identified and trained. Infection control procedures will need to be boosted in most settings. Specialized treatment centers will need to be replicated, since there will always be patients requiring specialized care. Recording and reporting systems for drug-resistant cases, integrated into those for drug-susceptible patients, will need to be introduced.

Technical assistance is key to implementing these changes. Ideally, it should be sourced nationally, or in the region, but may have to come from the international community if unavailable locally. Experience from laboratory strengthening suggests that on-site assistance over several months is more effective than repeated “helicopter” visits of experts to ensure local capacity building and real transfer of knowledge.

Global guidance on MDR-TB management exists [19], but additional work is required (eg, on human resource policies, involvement of the private sector, and community care). Getting results to the patient rapidly is so important for cases with MDR-TB, especially if coinfected with HIV, that an international consultation is overdue to determine the role of modern information technologies such as mobile phones, which have already shown value in antimalarial drug management [20].

**WHAT DOES THE WORLD REALLY NEED?**

The escalation in TB diagnostic tool development over the last decade presents real hope for addressing one of the main barriers in TB care and control. Nevertheless, the ideal POC test still eludes us and may remain an impossible dream without significant innovation in research and funding strategies, tempered by the realization that no single test could probably meet all ideal requirements. Therefore, in the foreseeable future, new tools in the pipeline need to be rapidly assessed and deployed if found to be good, while implementation of existing tools has to be accelerated within revised diagnostic algorithms and at the appropriate levels of laboratory services. One size no longer fits all, and policy reform needs to become dynamic and responsive to a rapidly changing, technology-driven world.

However, new diagnostics per se would not have the public health impact needed to reduce, let alone eliminate, TB. Any diagnostic test is only as good as its access and utilization, and the best-performing diagnostic test will fail if patient- and health-service delays are not addressed in tandem. More focused research in diagnostic trials is needed on patient and health service impact, while uptake of new technologies must be facilitated by addressing patient and health service barriers.

Finally, diagnostic tools without treatment constitute an untenable situation. Therefore, as we capitalize on the technological advancements of the 21st century, the necessary political commitment to treatment would need to be scaled up in similar fashion. Current tools can and should be used better. Closing the gaps between diagnostics, drugs, and delivery of patient care is what the world really needs, while waiting for science to deliver the next wave of new technologies.

**Notes**

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