



Research paper

Improving diagnosis and case management of patients with tuberculosis: A review of gaps, needs and potential solutions in accessing laboratory diagnostics



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ABSTRACT

Tuberculosis (TB) recently surpassed HIV/AIDS as the deadliest infectious disease. Despite some advancements in technologies and programs, TB remains a global health epidemic that, without significant gains in better diagnostic and management, will not abate. This review describes the molecular diagnostic gaps, needs, and potential solutions for improving access to diagnosis and management of patients with drug susceptible and drug resistant TB. The ultimate goal is to improve laboratory diagnostics and better individualized management of detected TB, and make an important contribution toward eliminating this global epidemic.

Rationale: Tuberculosis persists as one of the world's most deadly infectious disease. To make substantive progress toward TB elimination and end the global tuberculosis epidemic by 2035, innovative approaches and technologies are essential. Among the various related and complementary scientific and clinical milestones toward elimination, including vaccine development and new therapeutic regimens, one of the greatest challenges is to address the gaps in existing diagnostics. Current diagnostic technologies fall short in meeting the complex nature of the disease and its epidemiologic profile. This review concentrates and outlines the priorities for development of laboratory diagnostic tools for screening and diagnosing TB.

Approach: The approaches and priorities described in this manuscript are based on identified gaps of current laboratory diagnosis of different clinical forms of TB. Closing these gaps will enable national programs to more efficiently diagnose TB, monitor treatment efficacy, and control drug resistance. Prioritization is based on technologies that address facilitate a more patient-centered approach, and are appropriate for low-resource and high-burden TB settings. New diagnostics must complement current or anticipated developments elsewhere and accelerate increased coverage, quality, and impact at the lower levels of the healthcare system.

1. Introduction

1.1. The global burden of tuberculosis

Tuberculosis (TB) remains a global epidemic, recently surpassing HIV/AIDS as the deadliest infectious disease (Anon., 2016a). Recent advances in diagnostics and treatment of TB have had a tremendous impact, saving 49 million lives between 2000 and 2015, resulting in a 22% decline in the number of TB deaths (Nahid, 2006). Despite this progress, there are still 10.4 million new individuals developing active TB and 1.4 million deaths every year. The situation is further complicated among those living with HIV; 1.2 million people living with HIV

develop TB, with 400,000 deaths annually. Additionally, national TB programs face the multidrug resistant TB (MDR-TB) crisis with the threat of 480,000 new MDR-TB cases and an alarming 190,000 thousand associated deaths each year (Anon., 2016a).

These persistent challenges have shaped the global TB agenda, underscoring the need for more effective and scalable interventions to reduce and eventually eradicate the disease. The END TB Strategy (Anon., 2014a), led by the World Health Organization (WHO), aims to end the global TB epidemic by reducing TB deaths by 95% and to cut new cases by 90% between 2015 and 2035, through three pillars: i) integrated, patient-centered TB care and prevention; ii) bold policies and supportive systems; and iii) intensified research and innovation.

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This patient-centered approach of pillars indicates a long waited major paradigm shift in WHO's policy to tackle the epidemic of TB.

Improving diagnostics is a key part of Pillar 1 that comprises four components:

- Early diagnosis, including universal drug susceptibility testing and systematic screening of contacts and high risk groups.
- Treatment of all people with TB including drug resistant TB and patient support.
- Preventive treatment of patients with high risk, and vaccination against TB.
- Collaborative TB/HIV activities and management of comorbidities.

While we recognize the importance of development of new vaccines, drugs and treatment regimens, along with novel approaches of programmatic management of the diseases, this review focuses specifically on improving laboratory screening and diagnosis of the disease.

1.2. Premises for bringing high impact innovative approaches and technologies to end TB

Bringing new tools to end TB requires a systematic approach based on various premises:

- Tuberculosis elimination and eradication is achievable.
- New diagnostic tools and broader access to diagnosis will be necessary to accelerate progress toward slowing and elimination of the TB epidemic.
- A combination of phenotypic and molecular diagnostic approaches is necessary to address the complexity of the disease and on-going evolution of the pathogen.
- National TB programs will need to become more independent regarding external technical and financial resources, developing national strategic plans with the most practical and effective diagnostic algorithms as a mean to cost reduction and increased access to appropriate TB control services.
- TB program prioritization may reduce as disease burden reduces prior to elimination.
- Technologies should preferably be platform-based, offering integrated testing benefits that include other diseases.

1.3. The disease pathway: the need for an effective combination of diagnostic tools

TB is not a homogeneous entity; it is both stubborn and complex in that it encompasses a diverse spectrum of disease between infection, activation, and transmission. As such, to accelerate efforts in reduction and elimination of TB will require a careful combination of individual diagnostic solutions that are effective, as well as a tiered combination of diagnostic tools. Even within the lungs of a single individual, multiple lesion types coexist and individual pulmonary lesions can change substantially over time (Kaplan et al., 2003; Kim et al., 2010). Human TB is caused by *Mycobacterium tuberculosis*, a bacterial species within the *M. tuberculosis* complex, the diversity and behavior of which have enabled *M. tuberculosis* to be a very “successful” human pathogen (Kaplan et al., 2003), its unique characteristics and its genotypic and phenotypic plasticity allowing the bacteria to evolve and adapt to a challenging host environment (Fig. 1).

For example, a single mutation in the promoter of the dormancy survival regulator (DosR) regulon is the sole requirement for the dramatic shift in the pattern of dormancy related functions of *M. tuberculosis* strains of the globally important Beijing lineage (Chauhan et al., 2011). DosR regulon is associated with the metabolic adaptation to the host environment and the mutation potentially confers a fitness advantage in the face of some form of host-associated selective pressure (Leistikow et al., 2010). While the regulon requires induction by

conditions that inhibit aerobic respiration in most *M. tuberculosis* isolates, its expression is uncoupled from the need for signaling in members of the Beijing lineage (Chauhan et al., 2011). Similarly, many strains with drug resistance-associated mutations can activate compensatory mechanisms that restore their fitness and can significantly influence the spread of these particular strains in the population (Leistikow et al., 2010). These and many other important phenotypic differences between distinct *M. tuberculosis* lineages and strains have the potential to impact the efficacy of diagnosis, vaccination and treatment programs making clear that no standalone conventional or novel biomarker or molecular-based technique will be able to address all the diagnostic needs in itself. Instead, diagnostics must be designed and organized into a complementary and tiered approach that can significantly impact case detection and management that spans the spectrum of the following needs: triage testing of individuals with presumptive TB, detection of new cases, more complex but universal testing for drug susceptibility, and treatment monitoring. This suite of diagnostic solutions must also account for the complexity and throughput based on the level of care within the health system, which may vary between the primary care or referral center and therefore have different capabilities and resources. Early detection and adequate management of patients with all types of TB will depend on how correctly the different methods are used concurrently and be reliant on an appropriate laboratory network, and innovative, but emerging, methods of connectivity and decision support.

Of particular importance in the global fight to end TB relates to containing and reverting the drug resistant TB epidemic, wherein *M. tuberculosis* is subject to the growing problem of antimicrobial resistance (AMR) affecting a wide range of infectious agents, and as such is a broad public health threat. Drug resistant TB is having a huge negative impact worldwide and accounts for more than 1 in 4 AMR infection fatalities per year (Anon., 2014b). The average cost of just the drugs for treating the drug resistant TB patient can be 50 to 200 times higher than the cost of treating a drug susceptible TB patient (US\$ 100–1000 for drug-susceptible TB versus USD \$2000–\$20,000 for MDR-TB). This cost is particularly burdensome for high TB burden low and middle income countries (LMIC) countries, where up to 50% of the TB allocated resources may be spent on this patient group alone. Correct diagnosis and timely treatment of these individuals is essential to prevent further transmission of drug resistant TB and perpetuation of the global TB epidemic.

2. Recent approaches and their impact on ending the global TB epidemic

In the absence of a 100% effective vaccine for TB with which 25% of the global population is currently infected, halting the TB transmission chain and progressing toward a world free of TB must concentrate on early detection and adequate management of every individual developing the disease (Fig. 1). The complexity of diagnosing *M. tuberculosis* is reflected in Fig. 2 below, capturing the need to detect and distinguish latent TB infection (to prevent progression to active TB), active TB disease, drug resistant TB and response to treatment. None of these can be achieved without an affordable and effective diagnostic component that includes an efficient laboratory network and readily accessible point-of-care testing.

An effective diagnostic laboratory must go beyond the shortcomings of the oversimplified “one size fits all” directly observed treatment short-course (DOTS) TB control strategy. Under DOTS, only patients with acid-fast bacilli (AFB) microscopy positive sputum were treated (Anon., 1999), assuming that only these patients posed a major public health threat. DOTS patients were easily diagnosed through smear microscopy, which is accessible for most patients. However, DOTS has failed to account for the low sensitivity of smear microscopy and therefore resulted in underdiagnosis. It became clear that a new low-cost screening technology to replace smear microscopy was needed at

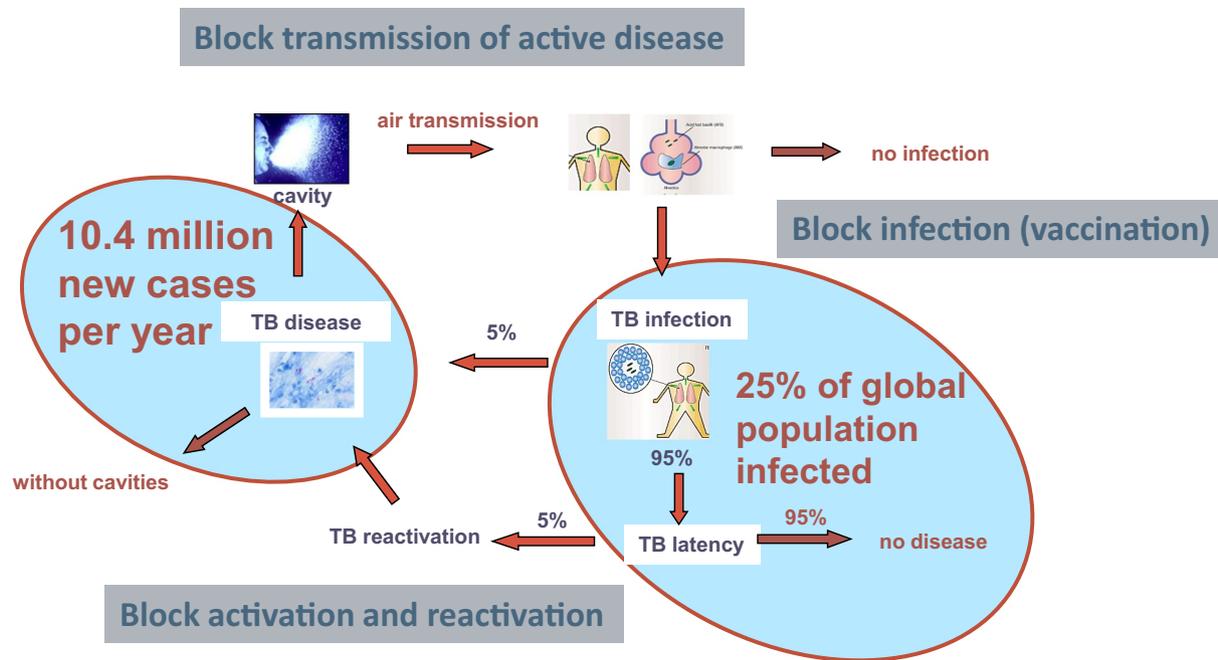


Fig. 1. The life cycle of *Mycobacterium tuberculosis* and its relation with the host.

Diagnosis and its purpose	Patient-centered				TB bacilli-centered				
	immune response		clinics	X-Ray	phenotypic		Molecular/ biomarker		
	humoral	cellular			microscopy	culture	DNA/RNA	Ag	MA/prot
Latent TB infection - risk of TB disease - treat latent infection	IGRASs ESAT6 CPF10	PPD (+) if BCG, NTM	NA	Only Gohn complx	NA	NA	NA	NA	NA
TB disease - to treat disease - IC measures			Presumptive diagnosis		NTM are (+)	Gold standard	Gold standard	LAM in urine	Only from culture
Drug resistance - which drugs to treat - for how long to treat	NA	NA	NA	NA	NA	Gold standard	Presumptive	NA	NA
Response to treatment - correct treatment ? - IC measure	NA	NA			Non-viable bacilli can be (+)	Gold standard	Non-viable bacilli can be (+)	LAM in urine	Only from culture
TB bacilli genotype - virulence - false positive - epidemiology	NA	NA	NA	NA	NA	Only to support DNA tests	Gold standard	NA	NA

Fig. 2. Existing biological approaches to diagnosing TB. “Patient-centered diagnosis” refers to examination of the patient’s response to infection (clinical, radiological or immune responses) whereas “TB-bacilli centered diagnosis” refers to macroscopic, microscopic and molecular examination of TB bacilli present in the clinical samples of the patient. No single diagnostic method is 100% sufficient to detect all TB cases. Early detection of TB will depend on how the different methods are used concurrently. (IC: infection control; NA: not adequated; NTM: non tuberculous mycobacteria).

the community level, to improve access to diagnosis much closer to patient populations. A second weakness of DOTS has been that both culture and drug susceptibility testing (DST) were restricted only to cases that were still AFB microscopy positive after two months of treatment to confirm viability and rule out drug resistance, assuming that only a limited number of these cases would be drug resistant. This lack of information on the drug susceptibility patterns and resulting inadequate treatment and follow-up of TB patients has played a key role in contributed to the global epidemic of drug resistant TB.

The urgency of developing, optimizing and introducing novel TB diagnostics is accelerating substantial progress in research and development of new TB diagnostic tools over the last decade. A variety of new techniques are shaping the field: light-emitting diode (LED) fluorescence microscopy for more sensitive smear microscopy; liquid culture for rapid *M. tuberculosis* complex detection and DST; molecular line probe assay for rapid screening for MDR; and fully automated, cartridge-based nucleic acid amplification assay GeneXpert MTB/RIF to detect drug susceptible and rifampicin-resistant TB.

These novel TB diagnostic technologies have contributed significantly to the improvement of TB control in low-burden high-income settings, which are now progressing toward TB elimination. In these settings, which have clear advantages in economic and infrastructure resources, it has been easy to support adoption and utilization of new technologies and provide adequate personalized treatment to each individual with TB. In LMICs however, the availability and scalability of these new diagnostic tools has been much slower; many of these high-burden settings continue to rely heavily on smear microscopy.

Key barriers to implementation and routine utilization of novel diagnostics in LMICs can be attributed to weaknesses in sample referral/transportation systems which impede scalability. Other challenges include low implementation rates for planned non-procurement related activities, inadequate equipment maintenance plans, low utilization rates and scaling of existing equipment such as GeneXpert due to low staffing levels. Expensive infrastructure requirements are necessary to support existing liquid culture technologies, a significant financial burden for countries who are then forced to limiting their use to central and district/regional levels.

There have been high expectations for the GeneXpert MTB/RIF technology (Cepheid, Sunnyvale, California, USA), a fully automated closed system that performs sample preparation and real-time PCR, and producing results in < 2 h. This system is capable of detecting *M. tuberculosis* complex while simultaneously detecting rifampin (RIF) resistance (targeting the *rpoB* gene) (Anon., 2014c). While GeneXpert has made some important contributions, it doesn't fully address all the requirements for high-burden LMICs to achieve the greatest impact and cost-effectiveness (Hanrahan et al., 2015). The test is primarily sputum-based and the specimen preparation is adequately simplified, to work effectively with raw sputum. However additional specimen collection is needed to confirm drugs resistance by growth detection (because pre-treatment for GeneXpert testing kills *M. tuberculosis*). The technology has the advantage of being cartridge-based but the reagents are temperature sensitive. Despite the fact that the Foundation for Innovative New Diagnostics (FIND) negotiated a price for a four module machine at approximately US\$ 17,000 and the Xpert MTB/RIF tests cost approximately US\$ 17 per cartridge (Albert et al., 2016; Lemaire & Casenghi, 2010; Van Rie et al., 2010), these prices are still high and the instrumentation requires additional expensive calibration and maintenance. In addition, the throughput is inadequate for high-burden LMICs (12–14 samples per working day) and the External Quality Assurance has not been adequately addressed.

The test presents adequate sensitivity on smear positives, and acceptable sensitivity on smear negatives, with a good specificity to detect *M. tuberculosis* complex. However, the performances for detecting RIF resistance show various problems such as lack of detection of certain forms of acquired drug resistance because the system needs the mutated population to be almost 100% to detect the mutation, the prediction of

RIF resistance in paucibacillary samples and for a few *rpoB* mutations types has resulted in both false-positive and false-negative results, and there is no information on the type of mutation (Chakravorty et al., n.d.; Hanrahan et al., 2015). Variables that may affect the tests overall performance include HIV prevalence, strain diversity, prevalence of specific drug resistance-conferring mutations, patient-related diagnostic delays and default rates (Van Rie et al., 2010). The GeneXpert MTB/RIF assay has the potential to be used in moderately equipped laboratories, however, it is unlikely to be used as a Point of Care (POC) diagnostic test in most peripheral settings, primarily due to poor infrastructure and limited resources. To date, it has been mainly implemented at district level with low penetration in the tiered diagnostic network; its impact has been moderate, increasing the number of confirmed cases but not the number of new cases detected (Hanrahan et al., 2015).

Line probe assays (LPAs) were endorsed by the WHO in 2008 for molecular detection of drug resistance from smear positive patients at risk of MDR-TB (Anon., 2008). Two available commercial LPAs are currently recommended by WHO: the Geno-Type MTBDR plus test (Hain Lifescience GmbH, Nehren, Germany) and the Nipro Assay (Nipro Corporation) (Pai et al., 2009). Hain Lifesciences specifically designed to test for resistance to second-line anti-TB drugs (fluoroquinolones, ethambutol, aminoglycosides and cyclic peptides), and which can be used in combination with the Geno-Type MTBDR plus test to identify XDR-TB (Brossier et al., 2010; Hillemann et al., 2009; Kiet et al., 2010). WHO analysis of systematic reviews and meta-analyses showed that LPAs are highly sensitive (97%) and specific (99%) for the detection of RIF resistance, alone or in combination with isoniazid (INH) (sensitivity 90%; specificity 99%), on isolates of *M. tuberculosis* and on smear positive sputum specimens. The major advantage of LPAs is that they can be performed directly on smear positive sputum samples, giving rapid (approximately 5 h) drug susceptibility results without the need for culture. However, the disadvantages of LPAs include being labour-intensive and requiring highly trained personnel and dedicated laboratory space and equipment (Nicol, 2010). This makes deployment of LPAs a challenge in high proportion of TB diagnostic centers within LMICs.

Recently, WHO conditionally recommended the use of loop-mediated amplification (LAMP) as a low complexity nucleic acid amplification test suitable for use at microscopy and higher-tiered test facilities to diagnose pulmonary TB in adults (Anon., 2016b). TB-LAMP is an isothermal manual DNA amplification with sensitivity higher than for smear microscopy (ranging 77.7% to 80.3%). Among smear microscopy positive patients, TB-LAMP sensitivity ranges from 95.2% to 96.6% across studies, depending on the reference standard used. The specificity of the assays ranges from 97.7% to 98.1%. This assay requires lower biosafety level and the equipment is more affordable than GeneXpert although it still requires electricity and temperature control. Limitations of TB-LAMP include i) it cannot distinguish drug susceptible from drug resistant TB, then it should not replace the use of rapid molecular tests that detect TB and resistance to RIF, especially among populations at risk of MDR-TB; ii) it cannot replace smear microscopy for treatment monitoring; and iii) it is unclear whether it has additional diagnostic value over smear microscopy for the testing of HIV positive patients. Thus, it is not recommended implementing TB-LAMP in settings with high rates of MDR-TB or HIV, or in any setting that is already effectively using GeneXpert.

Whole genome sequencing (WGS) provides a rapid and comprehensive view of the genotype of *M. tuberculosis* and holds great potential for the rapid diagnosis of drug-resistant TB within a clinically relevant timeframe. Work started recently to intensify on if WGS could provide an alternative tool to replace phenotypic DST and to be applied directly on sputum, and optimized to a field deployable format. WGS provides additionally the highest resolution when investigating outbreaks (Satta et al., 2017; Walker et al., 2017). However, several challenges remain to be solved before WGS can be routinely used in clinical practice and outbreak investigation to guide decision-making, which does not seem

Table 1

Major Gaps in Existing TB Diagnostics (TB: tuberculosis; LTBI: latent tuberculosis infection; POC: point of care; POT: point of treatment; DST: drug sensitivity testing; TAT: turn around time.

Situation	Major technical gaps in existing TB diagnostics
Latent TB infection	<ol style="list-style-type: none"> 1. Biomarkers of progression from LTBI to TB disease 2. Differentiation of those with imminent TB disease from other stages of LTBI
Active TB disease	<ol style="list-style-type: none"> 1. Adequate screening or case detection at POC and POT 2. Increased sensitivity and specificity: paucibacillary (children and immunosuppressed) 3. Non-sputum based, simple, cheap
Treatment monitoring	<ol style="list-style-type: none"> 1. Faster and less complex, decentralized 2. Total viability count 3. Persists/viable but not culturable 4. Viability but non-replication dependent
Drug susceptibility	<ol style="list-style-type: none"> 1. Reliable molecular DST 2. Informative, faster and less complex phenotypic DST, decentralized 3. New drugs, monitoring of new regimens
TB bacilli genotype	<ol style="list-style-type: none"> 1. Cheaper and automated systems for whole genome analysis 2. Directly on clinical specimen 3. Patient management vs. surveillance
Specimen referral and reporting	<ol style="list-style-type: none"> 1. Mobilize samples when patient or diagnostic are not accessible 2. Shorten TAT for reporting to care giver

to be feasible in the near future. In particular, it is still unanswered how to deal with i) the complexity, throughput and cost of the technology (hardware and software) to perform the tests and to analyze the data; ii) the current discrepancies between genotype and phenotype; iii) the need for large data sets to clarify the role of rare resistance mechanisms and the level of resistance conferred by different mutations; iv) some of the current problems of traditional susceptibility testing, in particular pyrazinamide sensitivity; v) the heteroresistance; vi) the lack of understanding on phenotypic consequences of mutations and compensatory mutations on drug resistance not only on new drugs but also on several of the old TB drugs; and vii) the lack of understanding of these genetic alterations in different geographic settings with different *M. tuberculosis* lineages/clades. It will be also necessary to solve the need for direct sputum applicability and to optimize for lower levels of the tiered health laboratory systems.

Table 1 below captures major technical limitations in existing TB diagnostics. Whereas low-burden and high-income settings can overcome these limitations (accuracy, turnaround time, complexity) by combining the use of several different tests for each patient, the lack of resources with the complexity of several assays preclude similar success in high-burden countries, especially in LMICs. Collectively, the impact of these new diagnostic methods has been negligible. They have successfully resulted in more accurate diagnosis of the same number of patients with susceptible TB and MDR-TB that were previously diagnosed based on clinical suspicion and limited laboratory confirmation only (by classical smear microscopy and growth detection), but the anticipated high impact in the overall detection and management of TB cases in the field is moderate. As a result, about 4.3 million people living with TB (40% of all estimated TB cases) and 455,000 with MDR-TB (80% of all estimated MDR TB cases) are still missed by health systems every year (Anon., 2016a). In addition to causing excessive suffering and economic hardship, undiagnosed TB and MDR-TB cause sustain TB transmission and perpetuate the epidemic.

2.1. Importance of improved TB diagnostics for drug susceptibility testing in TB

A collateral effect of the lack of full (first and second-line drugs) and

detailed *M. tuberculosis* DST and of the complete lack of any DST for novel drugs for all patients detected as MDR-TB is inadequate management of harboring strains with additional resistances. This has resulted into treatment failure, uninterrupted TB transmission, and potential programmatic selection as well as development and emergence of even more resistant forms of TB such as the XDR-TB in South Africa (Müller et al., 2013). In the absence of information and quantitative DST to second-line drugs, only half of the notified MDR-TB cases are cured despite absorbing up to 50% of the TB resources allocated to this patient group alone (Anon., 2014a).

A systematic review of clinical outcomes of TB patients treated for different forms of drug resistance in LMICs has highlighted the implications of the oversimplification of present MDR and XDR classification. In the study, overall 62% of patients were successfully treated, in 7% treatment failed or the patients relapsed, 9% died and 17% defaulted (Falzon et al., 2011). Treatment success showed decreasing degrees from MDR-TB only to XDR-TB cases who had the lowest rates of treatment success and the highest rates of failure, relapse and death. This reality demands better access for TB patients in resource-constrained settings to laboratories which can perform DST reliably to detect resistance promptly and provide individualized treatment. Oversimplifying XDR may very well negatively impact the development, utilization, and application of new diagnostics. These risks are compelling factors to develop improved and field optimized diagnostic and case management tools that can achieve universal access in high burden LMICs and support strategies to preserve both existing and new treatment regimens. These strategies need timely and individualized treatment optimization to effectively control acquired or to avoid programmatically generated drug resistance.

3. Patient centered approach: Priorities for TB diagnostic development

In addition to the limitations described above, there are long-standing and persistent challenges with existing and novel TB diagnostics and their application in the global fight to end TB. Many of these shortcomings can be attributed to how and where they have been used, and the lack of specific and quantitative information all of which have considerable implications on treatment decisions and disease transmission. Ultimately, the global TB community must shift toward a more individualized diagnostic approach rather than “one size fits all” or “shotgun” approach that mistakenly misidentify TB latency or drug resistance. This will require diagnostics that can be deployed at lower levels of the health care system, through an integrated laboratory network, and be more targeted in specific and measurable information about *M. tuberculosis*. This is precisely the challenge in finding interventions that can curb transmission, facilitate more effective treatment pathways, and reverse the antimicrobial resistance (AMR) characteristics of this epidemic.

3.1. Remaining diagnostic gaps and their importance: what? where? how?

3.1.1. Enable prediction of progression to active TB

The natural history of *M. tuberculosis* is more complex than most bacterial pathogens and this is one of the keys of its success as a pathogen (Vynnycky & Fine, 1997). Transmission of *M. tuberculosis* results in only a small portion of infected people developing active disease in the short term, whereas 90% of infected people remain with latent TB that could become active at any time during one's lifetime. Recent estimates suggest that close to a quarter of today's global population has latent TB infection -around 1.7 billion people (Houben & Dodd, 2016). Over 80% of this burden is concentrated in Asia and Africa and concentrated in older age groups as the prevalence of infection increases with age, though nearly 100 million children (more than the entire population of Germany) already carry a latent TB infection. With progress toward controlling the active TB epidemic and TB elimination, it

will be necessary to identify new strategies to reduce the *M. tuberculosis* reservoir of latently infected individuals from which new cases arise. Providing preventive treatment to latently infected persons will be part of these strategies and predictive diagnostic tests identifying those likely to reactivate into active TB will yield great benefit.

The most useful predictive biomarkers will most likely be mycobacterial products or markers of host response identified within blood or urine or through skin testing (Esmail et al., 2014), or through breath testing and detectable at the transition of latent to active disease with subclinical and minimally active pathology or evidence of immunopathology. However, unlike vaccine, drug and diagnostic tests for active TB, no pipeline exists yet for predictive tests for identifying those individuals with LTBI that are more prone for activation of the disease and within a relatively shorter time frame (6–12 months).

The development of tuberculosis from infection to active disease is due to different predisposing and precipitating factors (Esmail et al., 2014). Following infection, there may be a critical period where fate of infection is determined with predisposing factors influencing this outcome like HIV coinfection, malnutrition, diabetes, alcoholism or pro/anti-inflammatory imbalance. The primary infection may be progressive in a small proportion of patients, and in those that control the primary infection, a proportion may eliminate TB or exert highly effective control and be at very low risk of reactivation. In the third group, control may be unstable, waxing and waning in response to a variety of precipitating factors; reactivation of TB is highly likely in this high-risk group. Precipitating factors leading to progression of disease may include HIV coinfection, anti-TNF therapy, malnutrition, vitamin D deficiency or viral infections. Prior to clinical TB presentation these individuals may pass through a subclinical phase of active infection which may last months. During this phase *M. tuberculosis* may be isolated by culture or pathology may be visible through imaging prior to symptomatic presentation. Therefore, developing diagnostic tools that target biomarkers produced along the pathway between TB infection and TB disease would be useful in identifying and directing appropriate interventions in those affected.

3.1.2. At point of care

Although some recently introduced novel diagnostic methods have increased the number of confirmed TB cases and provided more insight into the burden of the disease, they have not made a significant clinical and epidemiological impact globally (Huddart et al., 2016). One of the key reasons is because they still have not reached lower levels of health care systems closer to the vast majority of the patients. This is particularly relevant to LMICs where access at peripheral and primary care centers are important hubs for service delivery. Therefore, ending the TB epidemic will require remarkable increase of case detection, clinical care, and treatment efficacy in high burden settings through diagnostics and treatment as close to patients as possible. Most of the highest TB burden countries have invested in basic TB diagnosis (i.e. smear microscopy) and drug-sensitive TB treatment services at the primary health level (L1, microscopy centres or primary health centres) and higher. However, very few have any TB diagnostic or treatment services available at the most decentralized community or village health worker level (LO) or have DST capacity at decentralized L1 level. This limitation means that patients or samples are being referred, which in low-resource settings often results in long turn-around-time, loss to follow-up, and continued transmission of TB in the community.

Indeed, analyses of cascades of TB care show major gaps in the continuum of care, and might explain the persistently high and underestimated incidence of TB in some countries (Fig. 3) (Subbaraman et al., 2016). These patterns also underscore the importance of an integrated laboratory network system that spans the levels of the healthcare system through improved access and efficiency.

Broader access to new methods early in the patient pathway will yield greatest impact in terms of reducing TB incidence. Any action plan for increasing case detection must consider not only the number of

expected patients, but also a realistic projection of persons required to be screened in order to detect a single case of active TB (number needed to screen, or NNS). NNS present a high heterogeneity between population groups - to find one case of TB in select risk categories at a population level ranges from 2314 to 89 in low and high TB incidence settings, respectively (Anon., 2013). Among HIV populations, NNS ranges from 25 to 10 in low and high TB incidence settings, respectively. In order to provide patients adequate and appropriate treatment in case of resistance, toxicity or drug interactions, as is routinely done in high income countries, screening must address the total number of expected TB cases, not just expected numbers of patients with drug resistant TB within specific risk groups, which is why NNS becomes a very important driver in appropriate diagnosis and management.

Importantly, any increase in access to better diagnostics designed either to improve case detection or case management must be linked to reliable access to adequate treatment at the point of testing to prevent loss to follow-up and mitigate the negative parameters of the TB care cascade. Appropriate treatment must be maintained for a minimum of 6 months with multidrug therapy until 36 months in cases of complex resistant cases (Falzon et al., 2011). Patients should have proximity and access to rapid and effective monitoring for treatment efficacy, which will be a dramatic improvement from current methods that have long delays, often from 2 to 9 months, for detecting treatment failures. Such delays have the dual effect of reducing treatment efficacy and perpetuating transmission.

Reduced frequency of culture monitoring or replacement of culture by sputum smear even in the final 12 months of treatment could threaten early detection of failure (Mitnick et al., 2016). Compromise on method could lead to greater delays to treatment adjustments or adjunctive therapies among important subpopulations treated for MDR-TB, e.g. those with HIV co-infection or baseline negative sputum smears. Therefore, there is need for expanded global laboratory capacity for high-quality culture, in addition to smear microscopy and rapid molecular tests. More sensitive indicators of nonresponse, with shorter turnaround time, would further facilitate early detection of resistance and avoid poor outcomes.

3.1.3. Detection for all forms of active TB

Another challenge confronting novel TB diagnostic methods is that assays are primarily targeting sputum testing, sidelining patients who have a non-productive cough, paucibacillary or extrapulmonary disease such as children, pregnant women or HIV infected individuals, thereby excluding these patient groups from starting adequate therapy. Moreover, most recent implementation efforts focused on using these methodologies for screening for presumptive drug resistant cases only, and not the broader potential TB population. Consequently, the vast majority of patients, particularly with drug susceptible disease, remained completely unaddressed. Therefore, it is critical to revise the present approach and move away from targeting screening of drug resistant cases and address the potential cases of TB more comprehensively.

3.1.4. Tests for individualized diagnosis and treatment

Even the more recent assays (LPA or GeneXpert, liquid culture with a single screening concentration) for the screening for presumptive drug resistant cases or the monitoring of treatment are underutilized because the results they generate are reported or interpreted in an oversimplified manner (Nahid, 2006). Typically, these approaches identify strains from patients only as drug susceptible, drug resistant, MDR or XDR without trying to predict the phenotypic level of drug resistance, the presence or absence of cross resistance and the implications relative to achievable drug concentration.

This is best exemplified by case of a first line drug, isoniazid (INH) in which quantitative susceptibility testing verified that in the presence of mutations in the *inhA* gene, a low level of in vitro phenotypic resistance (at concentration 0.1 µg/ml) can be expected. This is way

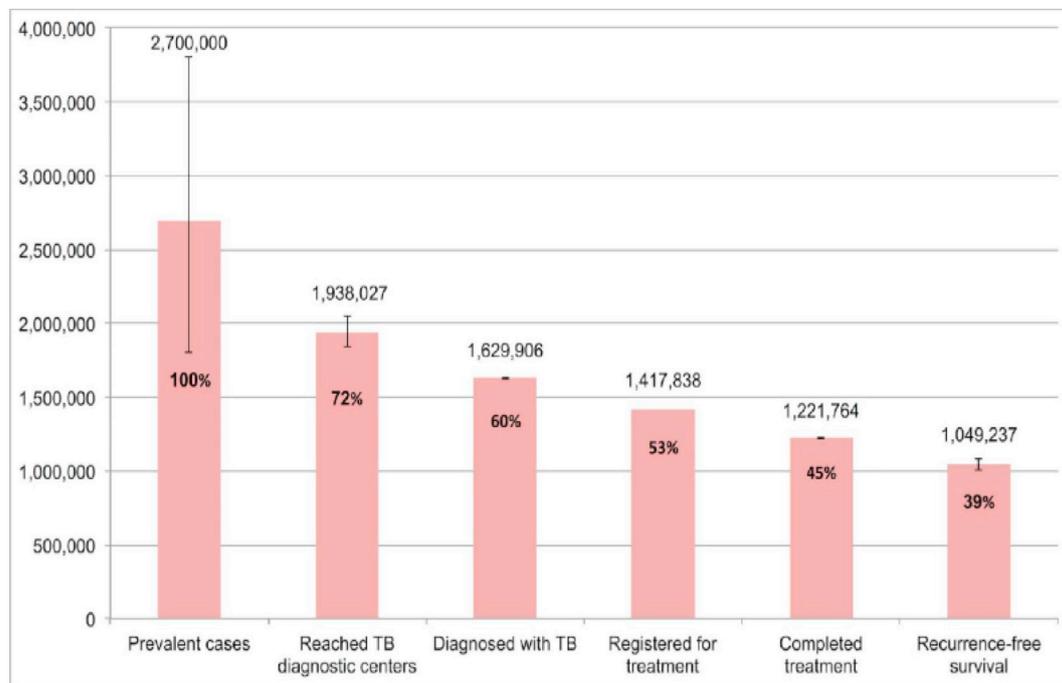


Fig. 3. Cascade of care of all forms of TB in India in 2013 (from Subbaraman et al. (2016) PLoS Med 13 (10): e1002149).

below the achievable serum concentration and therefore in vivo clinical experience shows that it can be successfully treated with increased dose of INH. In contrast, with mutations in the codon 315 of the *katG*, a high-level resistance can be expected, close to or above the achievable serum concentration. In this case dose adjustment is impossible without toxicity. Finally, mutations in other codons of the *katG* result in variable but moderate level of resistance, which may or may not be controlled by dose adjustment-commercial molecular tests usually do not identify them properly and their clinical relevance requires an individualized approach (Böttger, 2011). The present definition of MDR is resistance to INH at 0.1 µg/ml and rifampin at 1.0 µg/ml. If INH can be continued and the patient be cured with increased dosing, it is clear that to label these patients as having MDR is wrong from a clinical point of view.

Similar to the DOTS approach outlined earlier, novel diagnostic methods have also been used in a “one-size-fits-all” approach that lumps patients into boxes rather than using information to better understand the individual case and how to optimize treatment according to that individual. This is not a trivial weakness in existing diagnostics; it does not address susceptible cases but rather allows the continuous regeneration of drug resistance. It seems that the oversimplification of definition and rapid detection of drug resistance is also negatively influencing the suggested use of the newly developed short course 9-month Bangladesh regimen (Mitnick et al., 2016). This regimen is capable of decreasing the usual 18–24-month treatment in certain MDR-TB patients but it may already be in jeopardy as the available diagnostics in high burden drug resistant TB settings limit the ability to fully exploit the potential of this regimen (Aung et al., 2014). The reason is that TB programs are unable to readily identify and prioritize those patients who could best benefit from this treatment and avoid its use without proper additional adjustment in those who suffer more complex and specific forms of drug resistant TB. In addition, we do not have any reliable routine molecular or phenotypic DST to monitor and guard the long awaited newly available drugs such as bedaquiline and delamanid. It is not common knowledge that these new drugs are in use for treatment for more than 10 years without any clinical laboratory support.

3.2. Priority fields for tuberculosis diagnostic development

To adequately address significant components of the remaining diagnostic gaps described above, we propose the following priorities for improving TB laboratory diagnostics, with the primary goal to improve case detection and better case management of patients with both drug susceptible and drug resistant TB:

1. Identify patients with TB infection or subclinical form of the disease that are imminently or likely to develop active disease to prevent full disease activation and progression of advanced forms.
2. Identify much higher numbers of individuals with either presumptive TB or active cases at the point of care:
 - accurate detection of all forms of TB including extrapulmonary TB (detect and cure the 45% of all new TB cases currently missed by existing diagnostic approaches).
 - accurate detection of TB in specific groups with paucibacillary disease: HIV-associated TB and pediatric TB (detect and treat TB on the missed 65% of HIV (+) TB cases).
3. Rapidly identify drug susceptible and drug resistant (including MDR and XDR-TB) cases for early optimization of treatment upon initiation and prioritization for more detailed susceptibility testing (detect and cure the 80% of all new MDR-TB cases that are currently missed).
4. Develop molecular or phenotypic susceptibility testing to bedaquiline and delamanid for routine monitoring and early detection of potential emergence of resistance to these new drugs.
5. Improve monitoring of treatment efficacy (regardless of drug resistance).
6. Improve access to detailed and full scale DST to support individualized treatment (by facilitating information about level of resistance, cross resistance, implications relative to achievable drug concentrations, verification of novel assays) instead of just relying on rapid assays and standardized treatment regimens.
7. Bridge the access gap between patient and testing by improving transportation systems for specimens and results, particularly when complexity and throughput of technology alone cannot fulfill the needs of the point of care (diagnosis and treatment).

The focus for improvement should be on those approaches that best leverage the scope, capabilities, and cost and time efficiency toward a suite of effective TB diagnostic tools. These tools should be customized not only to the right level of a diagnostic system, but also have to be able to cut across diseases silos for better utilization of health care resources and to provide a patient centered service for several priority major diseases with public health concern as part of an integrated laboratory network, regardless the level of care. Approaches should strengthen these laboratory networks at different levels of service delivery in low-resource settings, and should maximize the access and scalability of effective TB diagnosis, as well as supporting diagnostic solutions for other diseases.

An integrated laboratory network can be defined as one that has the ability to provide all primary diagnostic services needed for the care and treatment of patients without requiring them to go to different laboratories for specific tests and indispensable for providing sustainable national support for global health initiatives for TB, HIV, malaria and other public health priorities (Somoskovi, 2015). To meet these requirements, the network should i) provide quality assured basic laboratory testing; ii) use common specimen collection, timely reporting and diagnostic platforms across diseases within the same facility; and iii) increase capacity for introducing and using new and more complex technologies. The extent of integration at the different levels of the laboratory systems should always be based on the complexity, throughput and specimen collection or referral requirements of the particular diagnostic platforms.

4. Potential TB diagnostic solutions that could be prioritized

We suggest the following key areas of development in which to focus activities collectively addressing important laboratory diagnostic challenges in the most cost and time efficient manner with the ultimate target in view. These key areas, novel biomarkers and specimen types, and use cases to be targeted are summarized in Table 2. More detailed information on every technology that is discussed in this paper can be found in the fifth edition of the Unitaid Tuberculosis Diagnostics Technology and Market Landscape report (Anon., 2017), that presents an updated and comprehensive overview of TB diagnostic technologies that are commercially available or close to market.

4.1. Case detection of all TB forms at point of care with management of drug sensitive TB patients

At the community level, where most of the population access health care services, laboratory assays that are rapid, simple, sensitive and accessible laboratory assays will significantly improve case detection and management. These assays should cover all types of active TB, optimally with accuracy that can support initiation of therapy of sensitive TB cases without referral, to decentralize point of treatment. There are three potential approaches to achieve this goal using existing capacities:

- A. *Developing a lateral flow assay (LFA)* or a similarly simple platform that is non-sputum-based (urine, blood, breath or sweat) but able to detect a single or small set of TB biomarker (e.g. TB lipoarabinomannan, volatile organic compounds) present in organic fluids or compounds. Like LFAs in use for other diseases, such an assay may be community-based and significantly increase access to diagnosis and the number of presumptive cases or patients identified.
- B. *Improvement of molecular assays* to develop a simple, affordable non-sputum (urine, blood, stool or saliva) biomarker-based (e.g. cell free DNA), point of care and/or point of treatment (optimally community, minimally microscopy center level) test that can support initiation of anti-tuberculosis therapy in patients identified by a referral test. This platform should offer the possibility to rule out MDR-TB (minimally) or to identify MDR and XDR-TB patients

Table 2
Priority potential solutions in addressing TB diagnostic gaps in the different phases of the TB process.

Diagnosis	Pre-analytical		Analytical		Post-analytical	
	Sample referral	Innovative specimen collection systems to preserve and/or increase yield	Automated reading and viability staining of AFB	Immuno capture of biomarkers LFA	Molecular testing for viable/nonviable TB	Phenotypic and growth detection
Latent TB infection	Improve	Immunative specimen transport approaches (UAVs)	Health care center	Community	Community/health care center	Host protein signatures
Active TB disease	- Access to testing - Throughput		Viability stains	MTB specific CD4/CD8 cell interferon gamma expression or T-cell surface markers	Molecular testing for viable/nonviable TB	Total viable count Quantitative (MIC)
Response to treatment Drug susceptibility	- Reflex testing of screening tools - Integration through platforms - Specimen quality, integrity Patient ID		NA	LAM VOCs or other novel antigen based biomarkers NA	Low cost incubator District/regional	
TB bacilli genome analysis	- Biometrics		NA	NA	Whole genome sequencing	NA

(optimally) aiding therapeutic decisions and guide referrals for treatment by predicting different levels of phenotypic resistance and cross resistances.

- C. *Improvement of an existing sputum-based technology for the tiered laboratory network* at the point of treatment such as AFB smear microscopy, using an automated and field applicable platform, that may significantly increase the number of patients (especially the most infectious ones) identified at the microscopy center level and also strengthen monitoring of therapy efficacy with the potential combination with viability detection (e.g. fluorescein diacetate or fluorescein labeled glycolipid staining), until more advanced tools become field deployable and sustainable.

4.2. Individualized case management with focus on the clinical implications of different forms of drug resistant TB cases

Faster, simpler, and more accessible phenotypic detection of the total viable count (including the viable but presently not culturable count) and quantitative drug susceptibility testing for decentralization (targeting regional and district level) can improve individualized case management, follow up and monitoring of treatment efficacy with focus on but not limited to treatment failure and different forms of drug resistant cases.

4.3. Innovative pre- and post-analytical approaches

- A. *Innovative specimen storage and transport systems* (e.g. unmanned aerial vehicles (UAV) with adequately temperature controlled low weight payloads and novel transport media to preserve specimen integrity or increase testing target yield) may be viable tools to further improve access to testing by moving the specimens to the diagnostic sites. While improvement or development of field-optimized temperature controlled specimen transporting systems and payloads.
- B. *Optimizing Specimen Preparation*. Quality testing requires quality specimens. At present many novel TB diagnostic assays are overlooking the problem of optimization of specimen preparation and often times using the leftover specimen prepared for growth detection. In order to improve the yield and to simplify the complexity of specimen sampling and preparation for TB testing, novel approaches are also indispensable in this pre-analytical field that allows to maximize testing performance of a particular assay and may even allow to use not only the same specimen type across all potential tests of a tiered network but preferable the same sample. Specific areas and approaches of improvement of specimen preparation to be addressed as part of a particular assay development:
- concentration of the target (e.g. filtration or capture systems)
 - extraction of the target (e.g. liquid biopsy systems from urine and blood)
 - stability of the target (e.g. chemical or temperature controlled)
 - removal of inhibitors (simplified homogenization, separation)
- C. *Appropriate patient identification using innovative biometrics* is key to reliable and up-to-date patient and not disease centered (multiple disease cross cutting) case management since TB treatment is long and the disease is often associated with other comorbidities such as HIV or hepatitis virus infection or non-communicable disease.

In conclusion, these potential novel approaches will not only need to be able to make a diagnostic and a therapeutic impact but in turn they must significantly improve patient outcomes such as improved loss to follow up, mortality and quality of life, and reduce transmission.

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