The Tuberculosis Diagnostics Pipeline

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INTRODUCTION

Diagnosing tuberculosis (TB) is the first step to being able to treat it and prevent transmission. New guidelines from the World Health Organization (WHO) note that diagnosis should be "available free of charge to all persons with TB and populations at risk."[1] Yet an estimated over four million people with TB went undiagnosed or unreported to national treatment programs in 2015; India, Indonesia, and Nigeria accounted for almost half of this gap.[2] Those who do receive a TB diagnosis often do so only after multiple health care visits and lengthy delays. Studies have found average delays of 28 to 30 days from when patients first contact a health care provider to diagnosis, even when patients present with overt TB symptoms.[3] Drug-susceptibility testing is not widely available and is used far too infrequently, even among those diagnosed with TB and living in countries with a high burden of drug-resistant TB (DR-TB). The end result is that 40% of people with TB do not receive a diagnosis or are not reported, and DR-TB is detected in only 23% of people thought to have it.

Ending this catastrophic neglect will require two simultaneous revolutions. First, we need dramatically increased ambition in and accountability for country- and local-level uptake of all the tools—including newly WHO-endorsed ones—required to adequately diagnose TB, detect drug resistance, and swiftly link patients to treatment (see TAG's *An Activist's Guide to Tuberculosis Diagnostic Tools*, www.treatmentactiongroup.org/tb/diagnostic-tools). WHO's plan to create a Model List of Essential Diagnostics, following calls from academics and activists, could help create such accountability.[4,5,6] Second, an infusion of investment into research to move forward basic science and the diagnostics pipeline is urgently needed.

We must secure the successful development of new diagnostic tests on the horizon that offer meaningful—albeit incremental—advances, as well as true innovations that could radically simplify and improve TB diagnosis. Towards the former, notable recent progress includes the launch of a more sensitive Xpert MTB/RIF Ultra assay for diagnosing TB and detecting rifampin resistance, further evidence of the effect of urine lipoarabinomannan (LAM) testing for people with HIV, a sputum LAM assay that could revolutionize treatment monitoring, and several rapid tests inching towards launch that could bring TB and rifampin resistance testing closer to patients (GeneXpert Omni, TrueNat) or expand susceptibility testing to more drugs (Xpert XDR, RealTime MTB RIF/INH, and FluoroType MDR). These and other advances are described below.

ADVANCES IN TB DIAGNOSIS AND DRUG-SUSCEPTIBILITY TESTING

GeneXpert Ultra

Perhaps the biggest advance so far this year in TB diagnostics has been the launch of the Xpert MTB/RIF Ultra assay (Ultra).[7] A WHO expert consultation found the Ultra cartridge non-inferior to the original MTB/RIF assay (which has still not been adequately rolled out, see textbox page 94) for diagnosing TB and detecting resistance to rifampin, based on data from a multi-center, 1,520 person study carried out by the FIND comparing the Ultra assay with MTB/RIF.[8] This study found that Ultra's overall sensitivity at 87.8% (95% confidence interval [CI]: 84.2 to 90.9%) was 5% higher (95% CI: +2.7 to +7.8%) than that of MTB/RIF's at 82.9% (95% CI: 78.8 to 86.4%). The highest increases in sensitivity were found in some of the previously most difficult to diagnose patients. In people with smear-negative, culture-positive TB, Ultra's sensitivity of 61.3% (95% CI: 52 to 70.1%) beat out MTB/RIF's of 44.5% (95% CI: 35.4 to

Table 1. 2017 Tuberculosis Diagnostics Pipeline: Products with New Published Data or Policy Updates since the *2016 Pipeline Report*

Test	Type	Manufacturer	Status
MOLECULAR/NAAT			
FluoroType MTBDR	Semi-automated direct MTB detection; PCR in a closed system; results in 3 hours	Hain Lifescience	CE marked, and launched for marketing April 2017; not yet evaluated by WHO
MTB Complex	RT-PCR	BioGx, runs on the BD Max automated platform	CE marked, and launched for marketing in Europe April 2017; not yet evaluated by WHO
TB-LAMP	Manual NAAT by loop-mediated isothermal amplification (LAMP) for MTB detection	Eiken	WHO guidance issued in August 2016[9]
RealTime MTB/TB MDx m2000	Automated RT-PCR for MTB detection; can be used alongside HIV RNA platform	Abbott	Average sensitivity 92.1%, (95% CI: 87.9 to 99.9%) in smear-positive and smear-negative samples[10]; not yet evaluated by WHO
TrueNat MTB	Chip-based NAAT with RT-PCR on handheld device for MTB detection	Molbio Diagnostics, Bigtec Labs	FIND and ICMR studies underway; submission for approval in India expected end of 2017; not yet evaluated by WHO
Xpert MTB/RIF Ultra	Next-generation cartridge-based detection of MTB + rifampin resistance	Cepheid	WHO guidance issued in March 2017[11]
GeneXpert Omni	Single-cartridge mobile platform for single Xpert MTB/RIF or Ultra cartridge	Cepheid	Platform under development. Launch expected third quarter 2017; not yet evaluated by WHO
Xpert XDR	NAAT cartridge for GeneXpert platforms that can detect resistance to isoniazid, fluoroquinolones, and the second-line injectable agents	Cepheid	Assay under development; not yet evaluated by WHO. Preliminary sensitivity and specificity (as compared to sequencing, and not yet peer-reviewed):[12] • isoniazid 98.1%, 100%; • fluoroquinolones 95.8%, 100%; • kanamycin 92.7%, 100%; • amikacin 96.8%, 100%
ANTIBODY/ANTIGEN DETECTION			
Determine TB LAM Ag	Urine dipstick for TB LAM protein	Alere	New studies show incremental yield (additional cases detected) and correlation of LAM positivity with mortality (further supporting previous evidence that LAM can be used to accelerate treatment start and reduce mortality);[13,14,15] included in GLI algorithm[16]

CE: Conformité Européenne (European Conformity, an indication of permission to market in Europe); GLI: Global Laboratory Initiative; ICMR: Indian Council of Medical Research; MTB: Mycobacterium tuberculosis; NAAT: nucleic-acid amplification test; RT-PCR: real-time polymerase chain reaction; WHO: World Health Organization

53.9%) by a difference of 17% (95% CI: +10 to +25%). In people with HIV, Ultra's sensitivity of 87.8% (95% CI 79.6, 93.5) was 12% better (95% CI: +4.9 to +21%) than MTB/RIF's of 75.5% (95% CI 65.8 to 83.6%). In a single small, prospective study of people with TB meningitis, Ultra detected 95% (21 of 22) of confirmed TB meningitis cases compared with only 45% (10 of 22 cases) of cases detected using MTB/RIF (P = .003). In a single study of 378 children, Ultra's sensitivity was 24% higher than that of MTB/RIF. Ultra can better differentiate clinically meaningful (i.e., rifampin-resistance conferring) mutations from 'silent' mutations than MTB/RIF (though it still doesn't detect all mutations that confer rifampin resistance, which is a growing problem, as screening with an imperfect test allows the population of bugs with mutations that are not detected to expand).

Ultra's increased sensitivity came at a tradeoff of decreased specificity, which at 94.8% (95% CI: 79.6 to 93.5%) was 3.2% lower (95% CI: –2.1 to –4.7%) than that of MTB/RIF at 98% (95% CI: 96.8 to 98.8%), especially in patients with a history of TB (difference: –5.4%, 95% CI: –9.1 to –3.1%). This is likely because Ultra detects non-viable bacilli. The WHO Report notes that "in low TB burden settings and in the testing of specimens for the diagnosis of extrapulmonary TB and paediatric TB, false positive results were not a major concern," and even a 'trace' result (a new, semi-quantitative category that corresponds to the lowest bacillary burden for detecting MTB) with Ultra is sufficient to start therapy in these populations and in people with (or thought to have) HIV. The remaining risk of overtreatment as a result of false positives in high-TB-burden settings can be mitigated by repeating the test on a fresh sample when Ultra reports 'trace' results in HIV-negative adults with signs and symptoms of TB.

The WHO supports Ultra as an alternative to the current MTB/RIF in all settings, and Cepheid, its manufacturer, will gradually phase out the current MTB/RIF assay and replace it with Ultra.

Omni

Cepheid is also slated to release another major technology improvement in the third quarter of 2017: the Omni. This portable, rugged, battery-operated, single-module (cartridge) instrument will enable the Xpert technology to reach 'level one' facilities (primary health posts and centers).[17] This could help to dramatically reduce time to diagnosis for many patients and facilitate the switch from the far less sensitive smear microscopy (though smear will still be needed for treatment monitoring), and help to detect rifampin resistance much earlier. However, as it only processes one sample at a time, it is not suitable for clinics with a high attendance of people needing to be evaluated for TB, unless many are purchased. The Omni is expected to cost about $2,700 per device.

Cepheid's original requirement of using Omni along with its connectivity software, C360, has raised important questions for the field about the ownership and parameters of use of data, whether countries allow data to be sent outside of the country (C360 hosts data on an external server based in the U.S. and U.K.), and whether third-party connectivity solutions can be used. The Global Laboratory Initiative is expected to put out a guide to connectivity solutions soon after this report goes to press.

Xpert XDR assay

Ultra enhances the GeneXpert system's ability to detect TB and rifampin resistance, and Omni aims to bring GeneXpert technology closer to where people seek care, but neither expands the drugs for which the Xpert system detects resistance beyond rifampin. That's where the XDR cartridge—sometimes referred to as the Xtend XDR—in development by Cepheid with support from the U.S. National Institutes of Health (NIH) comes in. According to data that have not yet been peer-reviewed, the test showed promising early results detecting resistance to isoniazid (sensitivity 98.1%, specificity 100%), fluoroquinolones (sensitivity 95.8%, specificity 100%), kanamycin (sensitivity 92.7%, specificity 100%), and amikacin (sensitivity 96.8%, specificity 100%) as compared with sequencing (accuracy is worse when compared

with drug-susceptibility testing by culture, the gold standard).[18] Now it is just a matter of transferring this complicated test into a cartridge so it can work on either the GeneXpert or Omni system, and scaling up for manufacture. But this has been unacceptably delayed as a result of inadequate investment. Cepheid and Danaher, which acquired Cepheid, have indicated that Cepheid is working with partners to develop the XDR cartridge to prepare for its market launch.[19] But the funding gap means that it will not be available until 2019 at the earliest.[20]

The ability to rapidly and simply detect resistance to these drugs would be of tremendous value in quickly guiding the initiation of appropriate therapy. As new data show poor outcomes for treatment of isoniazid-monoresistant TB using the standard first-line regimen, a rapid test to detect isoniazid resistance is even more important than previously thought.[21] This could mean an even bigger market for the XDR cartridge than originally anticipated. However, using the full XDR cartridge on all TB-positive, rifampin-susceptible samples would be very expensive. It would also provide difficult to interpret and potentially undesired results about resistance to the injectables and fluoroquinolones, as running the assay in a population with a low prevalence of resistance to second-line drugs lowers its positive predictive value. Global and national guidance will have to reflect carefully on how to ensure isoniazid resistance is appropriately detected without misusing resources or misdiagnosing people. Far more optimal would be to have the isoniazid-resistance testing on the same cartridge as MTB and rifampin resistance detection, but it is not currently feasible to fit all on one assay.

At least for the meantime, line probe assay (LPA) and liquid culture will continue to play important roles in drug-susceptibility testing. Until the XDR cartridge is validated and available, second-line LPA (guidance issued by WHO in May 2016) is the only relatively quick way to determine who is eligible to receive the WHO-recommended shortened regimen for MDR-TB.

Seven years into GeneXpert rollout, and still much more to do

Recent studies from southern Africa have shown that Xpert can have a valuable effect when integrated into a complete and functional system. A pragmatic study in South Africa and Zimbabwe randomized patients in communities with high TB/HIV prevalence to intensified TB case finding with either the Xpert MTB/RIF test (and, if HIV-positive, the Determine TB LAM urine test) or sputum smear microscopy. Thirty percent more patients with TB—as later confirmed by culture—were started on treatment in the Xpert group than in the smear group at 60 days (86% versus 56%, 95% CI: 9 to 50%; P = .0047). Using Xpert MTB/RIF in active case finding not only increased the proportion of patients starting treatment, but also reduced the time to treatment initiation from four days to one day (P = .0407), and reduced the proportion of patients treated empirically or by culture (12% versus 53%; P < .0001).[22]

A nationwide retrospective cohort study from South Africa, which has been at the forefront of the MTB/RIF rollout, revealed that the test helped reduce treatment delays (by 44 days from pre-Xpert rollout in 2011 to post-Xpert rollout in 2013, P < .001) and allowed for more MDR-TB to be detected and treated. However, there is still a large gap between diagnosis and treatment: in 2013, the proportion of patients with rifampin-resistant TB who had started treatment at six months was no different if they were diagnosed by Xpert or other methods (62%, 95% CI: 59 to 65% versus 64%, 95% CI: 61 to 67%; P = .39).[23] This points to the need to ensure that all patients diagnosed are started on treatment, and rapidly so. A recent

review article notes that "Xpert MTB/RIF will only improve patient outcomes if optimally implemented within the context of strong tuberculosis programmes and systems."[24]

Overall, the use of Xpert MTB/RIF still lags, despite having been WHO-recommended since 2010, and its current recommendation as the initial diagnostic test for all persons with signs and symptoms of TB.[25,26] In 2016, the public sector procured 6.9 million cartridges under concessional pricing, up slightly from 6.2 million in 2015. But this is still far below the number that would be needed to diagnose the more than 10 million people who fall ill with TB each year. India and Indonesia have plans to scale up access to Xpert MTB/RIF, and dramatically increased procurement of modules in 2016; together they accounted for nearly half of the 8,316 modules procured in the public sector.[27] Whether these important tests will actually reach those in need and aid in speeding up the start of treatment remains to be seen.

Reports from users indicate that the GeneXpert devices have a short life-span and often need replacing just after the warranty ends, suggesting a need to improve service and maintenance arrangements.[28]

GeneXpert's use for other indications, such as early infant diagnosis (for which its qualitative test has already been prequalified by WHO) and HIV load monitoring (which is undergoing prequalification analysis now) could help to encourage rollout of the test, efficiency, and integration of care if done collaboratively. However, as procurement and as care for different conditions are largely done in silos, this opportunity has not yet been fully exploited.[29] The WHO Global TB Programme and HIV Department are planning to put out an information note on considerations for adoption and use of multi-disease testing devices in integrated laboratory networks, and expanding early infant HIV diagnosis (which is happening with Global Fund and Unitaid funding) will offer an opportunity for negotiating better terms, such as for service and maintenance price structure, for both HIV and TB.

Alternatives to GeneXpert

When the WHO recommended Xpert MTB/RIF, many 'fast followers' were expected to debut shortly thereafter. Seven years later, we see that TB diagnostic development advances slowly, not unlike the slow-growing bacteria themselves. Many tests have been dropped, while others are being used in countries such as China, India, and Korea, without multicenter evaluation in different settings with diverse epidemiology.[30] BD's Max MDR-TB, Roche's Cobas TaqMan MDR TB, Akonni's TruArray XDR-TB, and Ustar's EasyNat MDR-TB are all in development with timelines for market entry at least a year out.

TrueNat

Of these alternatives, Molbio's TrueNat is the farthest along, with a large-scale, multicenter evaluation underway supported by the Indian Council of Medical Research. Early data show promise in terms of sensitivity and specificity.[31] Review for approval in India is expected at the end of 2017.[32] This battery-operated, low-throughput device would be an important competitor to Omni in India, where TrueNat's local production would mean major savings on shipment and import duties. But the platform's lack of full automation (it requires two precision steps) may make it less desirable elsewhere, unless cost is highly competitive.

RealTime MTB RIF/INH

Another noteworthy competitor is Abbott's RealTime platform, which is already in widespread use in central laboratories for HIV-1 load testing. The Abbott RealTime MTB (for MTB detection) and MTB RIF/INH Resistance assays are available and CE-marked (meaning the device complies with the European In-Vitro Diagnostic Devices Directive and can therefore be commercialized in the European Union)—although not yet WHO-evaluated. The latter is the first test to offer rapid isoniazid- and rifampin-resistance testing together on a high throughput system. This test is fully automated, from extraction to amplification and detection. The test's packing insert cites specificity of 97% (95% CI: 95 to 98%) among 359 culture-negative samples, and an overall sensitivity of 93% (95% CI: 89 to 96%) of 212 culture-positive samples, with 81% sensitivity (95% CI: 69 to 90%) in 63 smear-negative samples and 99% sensitivity (95% CI: 95 to 100%) in 149 smear-positive samples, as compared with culture.[33] Drug-susceptibility performance is also very good, with sensitivity of 94.8% and specificity of 100% for detecting rifampin resistance, and sensitivity of 88.3% and specificity of 94.3% for detecting isoniazid resistance. WHO evaluation of this and other high-throughput centralized platforms, such as BioGx on the BD Max automated platform, and other products by BD and Roche, is expected in the first quarter of 2018.

FluoroType MDR TB and XDR

Hain Lifescience's new FluoroType MTBDR test is a rapid molecular genetic test that—according to the company—can detect TB directly from sputum specimens.[34] The 'mostly' automated PCR-based process is more user friendly and faster (three hours) than Hain's existing LPA technology. The Fluorotype MTBDR test putatively detects resistance to rifampin and isoniazid simultaneously in either sputum or culture samples—peer-reviewed data are not yet available, but a publication is in progress. If successful, this would address—though only at higher laboratory levels—some of the challenges of lack of ability to diagnose isoniazid resistance along with rifampin resistance discussed under the Xpert XDR section. Hain launched Fluorotype MTBDR in April 2017, after it was CE-marked, and is evaluating what is needed for the test to be reviewed by the WHO in late 2017 or early 2018.[35] Hain notes they will have "very competitive, volume-based and market-adjusted pricing" for the product.[36] Development of an XDR product is expected to receive funding from an undisclosed European donor soon (we speculate the German government, given the company's location), and launch is anticipated in mid-2018.[37] Hain is also exploring ways to develop a pyrazinamide resistance assay—which, if developed, would be the first molecular test able to rapidly detect pyrazinamide resistance—using the Fluorotype platform, but would need funding to commercialize it.

LAMP

Not to be confused with LAM (TB seems to have a branding crisis alongside its diagnostic crisis), LAMP stands for loop-mediated isothermal amplification. WHO issued guidance for the use of TB LAMP, another molecular nucleic-acid amplification test (NAAT), as a potential replacement for smear microscopy in August 2016.[38] Similar to Xpert, LAMP is a NAAT, and is faster (about 40 minutes) and less expensive than Xpert.[39] TB LAMP is more sensitive than smear microscopy, with a sensitivity of 40.3% (95% CI: 27.9 to 54.0) to 42.2% (95% CI: 27.9 to 57.9) in smear-negative samples. However, the test cannot detect drug resistance, requires several manual steps, and WHO reported that it could not make recommendation about the use of TB LAMP in the detection of TB among people with HIV due to lack of data.[40] The test may be an improvement over smear in some settings with low rates of HIV and drug resistance, but is unlikely to bring major changes to the field.

LAM testing

New data further support the urgent need to introduce Alere's urine-based Determine LAM TB test, for which WHO issued guidance in 2015 as a rule-in test for people with HIV with very low CD4 counts (<100 cells/mm^3) or who are seriously ill. The test is particularly useful in hospital inpatient settings.

Researchers from the University of Cape Town—including Dr. Stephen Lawn, who unfortunately passed away in late 2016 after dedicating years of his life to the advancement of care for people affected by TB/HIV—conducted a study of 427 HIV-positive adults with acute medical hospital admissions, regardless of clinical presentation or symptoms. None were receiving TB care; 139 were later confirmed to have TB. In the first 24 hours of admission, sputum and urine samples were obtained from 37% and 99.5% of patients, respectively (P < .001). Sputum microscopy yielded just 19.4%. MTB/RIF using sputum yielded a slightly improved 26.6%. Urine LAM testing captured 38.1%, and combining MTB/RIF using sputum and urine LAM allowed for a 52.5% yield (P < .01). Urine LAM testing's value in improving yield was more dramatic in people with very low CD4 counts (<100 cells/mm^3: 18.9%, 24.3%, 55.4% and 63.5%, respectively; P < .01). The urine LAM test's yield was unrelated to respiratory symptoms, and specificity was 98.9% (274/277; 95% CI: 96.9 to 99.8%). A positive LAM status was strongly associated with death at 90 days (adjusted hazard ratio 4.20; 95% CI: 1.50 to 11.75). This clearly indicates that routine urine LAM testing for TB in newly admitted HIV-positive adults is feasible, provides major improvement in diagnostic yield with high specificity, is useful in identifying TB in people without respiratory symptoms and/or unable to produce sputum, and can rapidly identify patients at highest risk of death.[41]

A prospective observational study led by Médecins sans Frontières (MSF) in Kenya looked at the incremental diagnostic yield of urine LAM testing among hospitalized, symptomatic, and ambulatory (severely ill, CD4 < 200 cells/mm^3 or with body mass index < 17 kg/m2) HIV-positive adults.[42] Among 474 patients, 156 patients had confirmed TB—65.4% of them were LAM positive. Adding LAM increased the diagnostic yield of the algorithms from 47.4% (95% CI: 39.4 to 55.6%) to 84.0% (95% CI: 77.3 to 89.4%) when using clinical signs and X-ray; by 19.9%, from 62.2% (95% CI: 54.1 to 69.8%) to 82.1% (95% CI: 75.1 to 87.7%) when using clinical signs and microscopy; and by 13.4%, from 74.4% (95% CI: 66.8 to 81.0%) to 87.8% (95% CI: 81.6 to 92.5%) when using clinical signs and Xpert. Similar to the Cape Town study, LAM testing helped detect those at most risk of death: LAM-positive patients had an increased risk of two-month mortality (adjusted odds ratio: 2.7; 95% CI: 1.5 to 4.9).

In a third prospective TB cohort study—the first outside of Africa to our knowledge—researchers examined frozen urine samples from 109 patients with proven culture-positive TB for blinded urine LAM testing.[43] This is important as, unlike the sub-Saharan Africa setting that tends to have more advanced disseminated TB in the context of HIV co-infection, Thailand has more severely ill, disseminated, and pulmonary TB cases without HIV infection. The study included HIV-positive patients with TB; HIV-negative patients with disseminated TB; HIV-negative immunocompromised patients with TB; and diseases other than TB. The sensitivity of urine LAM in people with HIV was similar to that found in other studies (38.5%, 40.6%, and 45%, for CD4 T-cell/mm^3 counts >100, ≤100, and ≤50, respectively). LAM testing had an added effect in smear-negative, culture-positive people with HIV with disseminated TB with or without pulmonary involvement, increasing sensitivity to 44%. In HIV-negative patients with disseminated TB and in HIV-negative immunocompromised patients with TB, the sensitivities of the tests were 20% and 12.5%, respectively, and the specificity and positive predictive value were 100% for both groups. Positive urine LAM results were significantly associated with death.

Despite good results in such vulnerable populations, no country has yet committed to using LAM testing beyond pilot projects. The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) included LAM testing in its 2017 Country Operating Plan (COP) Guidance as a commodity that can be purchased using

PEPFAR's HIV/TB budget code.[44] The Global Laboratory Initiative included LAM testing in its updated TB testing algorithms.[45] Countries with high burdens of TB/HIV must immediately roll out the Determine LAM TB test in hospital settings for all newly admitted HIV-positive patients who are seriously ill, regardless of symptoms.

LAM for treatment monitoring

A separate technology focuses on the same antigen, LAM, which underpins the Determine LAM TB test. Otsuka (developer of delamanid and OPC-167832, see Marcus Low's TB Treatment Chapter on page 129) is developing a new enzyme-linked immunosorbent assay (ELISA) that quantifies LAM concentration in sputum. In a clinical study of 308 HIV-negative participants, this assay was found to be highly specific, correctly identifying 100% (95% CI: 94.8 to 100%) of 56 people without TB and 97.8% (95% CI: 92.4 to 99.4%) of the 92 people testing negative for TB with smear, culture, and GeneXpert, but had been clinically diagnosed as TB based on symptoms and chest X-ray. LAM-ELISA's sensitivity was better than smear, detecting all 70 smear- and culture-positive samples (95% CI: 94.8 to 100%), and 50% of 58 smear-negative, but culture-positive, samples (95% CI: 37.5 to 62.5%). However, LAM-ELISA's sensitivity was still less than Xpert MTB/RIF, which detected 79.3% (95% CI: 67.2 to 87.8%) of smear-negative, culture-positive samples.[46]

In a second study, LAM-ELISA was used to quantify sputum LAM concentration in 40 participants with smear-positive, pulmonary TB patients before treatment and at days 7, 14, 28, and 56 after starting standard treatment for drug-susceptible TB. LAM concentrations correlated strongly with time to detection in Mycobacterial Growth Indicator Tube (MGIT) liquid culture, and decreased during standard drug-sensitive TB treatment, indicating a potential use for treatment monitoring.[47]

Otsuka, working with the Critical Path to TB Drug Regimens, is developing sputum LAM as a biomarker for measuring treatment response as an alternative to microscopy and culture. Efforts are ongoing to seek qualification from the U.S. Food and Drug Administration and the European Medicines Association to use LAM as a new drug development tool or method.[48] This LAM assay, if further developed could have role for treatment monitoring in programmatic use. However, because the ELISA platform is cumbersome, the assay is currently complex and lengthy, and thus might not be suitable for use for monitoring TB outside of clinical trials. Further investment could allow it to be improved and modified for use in routine patient care. Otsuka and outside funders should collaborate to fully develop this potentially important assay for patient care.[49]

Liquid culture

Liquid culture remains the gold standard for diagnosing TB and detecting drug resistance. Automated and much faster than solid culture, it is particularly important for monitoring treatment response in people with MDR-TB, and would remain so even if Xpert XDR cartridges do successfully make it to market (since the latter cannot be used for treatment monitoring due to inability to distinguish between dead or live bacilli). Unfortunately, availability of MGIT automated liquid culture has been low, in part due to unaffordable pricing in places that were left out of a long-standing concessional pricing agreement (including some high-burden, low-income countries).[50]

In an effort to improve access, the test's manufacturer Becton Dickinson (BD), FIND, the Stop TB Partnership, and the United Nations Development Programme (UNDP) announced in March 2017 an expansion of the test's concessional pricing to include 40 additional low- and middle-income countries, making a total of 85 countries eligible for reduced pricing.[51] While a step forward, especially for countries like Papua New Guinea, many countries included are extremely small with low burdens of DR-TB. Many high burden countries (e.g., Ukraine and Brazil) or countries that want to use MGIT but cannot afford the high commodities cost (e.g., Tunisia) are still left out of this agreement, which perpetuates inequities through tiered pricing. BD should move towards a single low price in all low- and middle-income countries with a transparent, volume-based system for reducing price further once targets are met.

Sequencing

Liquid culture remains the gold standard for drug-susceptibility testing, as molecular tests—although extremely specific—are suboptimal in terms of sensitivity compared with phenotypic tests.[52] But culture is time-consuming and requires high biosafety level laboratories. This leads to many patients lacking access to susceptibility testing for second-line drugs—WHO recommends the use of five effective drugs when the shortened regimen cannot be used, but many people are unable to access appropriate treatment because their TB is not fully tested for susceptibility to second-line drugs to know which would work for them. The vision of universal, comprehensive, culture-free drug susceptibility testing can only be realized with sequencing. Whole-genome sequencing is already being used for surveillance, and in developed countries such as the U.S., all newly diagnosed TB cases have samples sent for sequencing. With lower cost, easy-to-use, next-generation sequencing forthcoming, sequencing could become much more practical and affordable than it currently is. Companies such as Illumina, BioMérieux, and Genoscreen are developing sequencing for TB, and ThermoFisher's Ion Torrent-based product is on the market for research use.[53] Pioneering work in high-burden countries has demonstrated the potential of using sequencing to guide treatment choice: in Mumbai, good data linking outcomes with specific types of *gyr A* mutations have been used to inform treatment decisions on fluoroquinolone choice and moxifloxacin dose based on the type of mutation seen. Similarly, being able to distinguish between *inhA* and *katG* isoniazid-associated mutations could help to define those in whom higher dose isoniazid might be helpful—those with *inhA* mutations and without *katG* ones. Similarly, mutations in the *eis* promoter region are known to predict resistance to kanamycin, and certain *rrs* mutations predict resistance to all aminoglyclosides.[54]

Sequencing could allow for a more sophisticated, individualized approach to treatment to ensure maximal efficacy without unnecessary side effects resulting from likely ineffective drugs. This approach will require much better and more rapid linkage between diagnostic results and patient care, as well as greater willingness for individualizing treatment than is currently seen with the preferred 'one size fits most' mentality in most TB programs. Direct sequencing from clinical specimens requires extracting DNA from MTB. This would require improved collaboration to rapidly define, optimize, and validate the best methodologies for sequencing MTB from samples.[55] Finally, sequencing can only be developed to guide individual treatment when better data exist to link mutations with patient outcomes. A recent assessment of five tools—CASTB, KvarQ, Mykrobe Predictor TB, PhyResSE, and TBProfiler—found false-susceptible results from drug-susceptibility testing were mainly due to missing mutations in the resistance catalogues that the respective tools employed for data interpretation, and that cases of false resistance resulted from the misclassification of polymorphisms as resistance mutations—pointing to the need for a high-quality catalogue of resistance mutations to ensure the clinical utility of new tools.[56] The ReSeqTB database is collecting such data and has an open call for contributions.[57]

Other advances in the detection of drug resistance

An important advance came in 2016 with the establishment of methodologies and minimum inhibitor concentration (MIC)[58] ranges for bedaquiline (0.015 to 0.12 µg/ml for the 7H10 and 7H11 agar dilution MICs and 0.015 to 0.06 µg/ml for the 7H9 broth microdilution MIC). However, these do not apply to MGIT, the commercial rapid liquid culture system. At the time of writing, WHO plans to publish in June 2017 an updated table of critical concentrations for second-line agents, including bedaquiline, clofazimine, and delamanid, for several culture media and MGIT. However, further research from larger data sets, such as one from Johannesburg, South Africa of approximately 1,000 patients, is needed to address concerns that the datasets informing WHO's guidance are too small.

The use of pyrazinamide—an important component of treatment of drug-susceptible and and some drug-resistant disease—also urgently needs better approaches for drug-susceptibility testing. This goal has remained elusive due to multiple resistance-conferring mutations all along the pncA gene, which codes for the protein that is pyrazinamide's target. Even drug-susceptibility testing on solid culture is challenging, as the acidic pH required to activate pyrazinamide impairs MTB growth. Sequencing of the pncA gene is likely the best way to determine resistance to pyrazinamide. As noted above, Hain is seeking funding to develop a pyrazinamide-resistance assay for the Fluorotype platform.

University of Maringá (Paraná, Brazil) researchers evaluated the resazurin microtiter assay (REMA) plate—an inexpensive, easy method that gives a colorimetric readout—at pH 5.5 for its performance in detecting susceptibility to pyrazinamide. They found that REMA was helpful for detecting pyrazinamide resistance when <50 µg/ml was considered as the cut-off, and results came in eight days. However, two known pyrazinamide-resistant isolates failed to grow at this pH level, indicating that it would be useful to evaluate this method at pH 5.6–5.9 to better understand REMA's utility in identifying pyrazinamide-resistant isolates.[59]

Moving forward by stepping back—antibody testing

Blood-based TB diagnostic tests have been inaccurate and unreliable, leading to the negative WHO recommendation against their use, and indicating that more research is needed.[60] A recent study analyzed IgG antibody responses to over 100 antigens in blood samples from 755 adults with presumptive pulmonary TB and found poor sensitivity for detecting TB (35% sensitivity at 90% specificity, as compared with a minimal target of 65% sensitivity at 98% specificity established by target product profiles).[61] A conventional antigen-based IgG detection test would therefore be unlikely to meet target product profile requirements, and does not merit further investment of limited TB R&D resources.

Chest X-ray

In 2016, the WHO issued a summary of its existing recommendations on chest X-ray as a screening tool for TB disease, indicating its sensitivity, its importance for diagnosing childhood TB, its additive value with GeneXpert, its use in diagnosing TB in people with HIV, and its role in ruling out active TB before treating latent TB infection.[62] Computer-aided detection (CAD), such as the CAD4TB software, may help X-ray technicians identify TB. The WHO will review the evidence and may make a recommendation in 2017 about the use of such computer-assisted reading tools.[63]

Improving TB detection through better sample transport

Another strategy for improving TB diagnosis in the field involves improving sample transport. The lack of a broadly accurate point-of-care test for TB leaves the field reliant on centralized testing and drug-

susceptibility testing, meaning that samples often have lengthy travels in suboptimal conditions to get from patient to lab. Over the course of storage and transit, the sample can degrade, making results less reliable. Reagents, such as cetylpyridinium, that have no need for a cold chain have been used in some settings for many years to stabilize sputum for higher quality following testing; however, its use is incompatible with culture. New commercial reagents aim to mitigate sample decay during sputum transport, improve convenience, and be compatible with culture.

OMNIgene SPUTUM is one such reagent; its sponsor says that it is compatible with culture and GeneXpert testing.[64] A recent study in Nepal of 100 samples, where transport time ranged from 2–13 days, handled samples in both the standard-of-care method and with new OMNIgene SPUTUM before submitting them for smear microscopy and GeneXpert MTB/RIF testing. The study found that overall smear results were comparable regardless of how the sputum was transported (58% in the OMNIgene group and 56% in the standard of care groups), but slightly more smear-negative samples were detected in the OMNIgene group (17% versus 13%; P = .0655, non-significant).[65] Another product, PrimeStore Molecular Transport Medium, by Longhorn, claims to be compatible with molecular testing (however, similar to cetylpyridinium, it cannot be used with culture as it kills the bacteria).[66] A WHO technical expert meeting in May 2017 reviewed the evidence associated with this and other sample transport innovations to advise whether these innovations are actually improvements or just more expensive; findings are expected by the end of 2017.

DETECTING LATENT TB INFECTION AND DISTINGUISHING IT FROM ACTIVE DISEASE

C-Tb

C-Tb is a new, specific skin test developed by Statens Serum Institute for two antigens, ESAT-6 and CFP10. The test aims to combine the advantages of older tuberculin skin testing, such as ease of use and inexpensiveness (and offers an alternative, as purified protein derivative used for tuberculin skin testing has been in shortage over the past few years),[67] with the specificity of interferon gamma release assays such as QuantiFERON. A double-blind, phase III randomized trial enrolled 263 individuals as negative controls, 299 occasional contacts of people with TB, 316 close contacts, and 101 people with TB disease. The study found that induration (the hard bump that develops, indicating a positive skin test) sizes were similar to traditional tuberculin skin testing, but C-Tb positivity, unlike tuberculin skin testing positivity, was not affected by BCG vaccination status. C-Tb and QuantiFERON testing agreed in 94% of participants over five years. Moreover, C-Tb test positivity trended up with increasing risk of infection, from 3% in negative controls to 16% in occasional contacts, to 43% in close contacts.[68] This test may help to better detect who is at most risk for developing active TB and in need of preventive therapy.

Quantiferon TB Gold Plus

Another approach to improving detection of TB infection is through improving the performance of interferon gamma release assays. The new-generation QuantiFERON test, QuantiFERON-TB Gold Plus, was recently evaluated in two studies. These showed that it has high concordance with its predecessor, and that the new test has a stronger association with surrogate measures of TB exposure in adults (such as average time spent with the index case).[69,70] The independent study authors indicated that the difference in interferon gamma production in the new test's two antigen tubes (TB2–TB1) can provide an indirect estimate of specific CD8 response, which correlates with increased MTB exposure, suggesting that it might be useful for identifying people with recent TB infection.[71]

RECOMMENDATIONS

Promising technologies are in development that can improve testing and simplify the current convoluted pathway to diagnosis. The little that has been invested in diagnostic development thus far has yielded impressive results. However, development time, and time to widespread uptake of tests, is taking far too long. With political will and resources, great advances can be made in reducing the unconscionable diagnosis gap. Interventions are critical in the following areas:

- **TB diagnostic tool development**: important advances such as the GeneXpert Omni and Xpert XDR assay have been moving too slowly through development, and others such as Otsuka's sputum LAM for treatment monitoring are at risk of languishing, largely because of inadequate investment in TB diagnostics research and development. In 2015, only $62.8 million was invested out of the $364 million required.[72] ***Increased private sector, public sector, and philanthropic investments in TB diagnostic R&D are urgently needed.***

- **Basic science research**: to move beyond sputum-based tests and all of their limitations, increased investment in basic science is crucial. Only with more investment upstream can we identify new markers of TB infection, disease, improvement, or worsening that could eventually underpin truly new, transformative diagnostic and treatment monitoring technologies. Yet basic science research in TB received just USD $139.8 million in 2015, out of the $455 million required.[73] ***Governments around the world and philanthropic institutions must increase basic science funding.***

- **Pricing**: in the current monopolistic market that diagnostic developers enjoy, pricing agreements are complex and vary widely between countries. As with drugs, a transparent, volume-based, flat pricing structure is needed for all TB diagnostics, including commodities and service and maintenance pricing. Given the distribution of resources and TB burdens, all low- and middle-income, as well as high-burden, countries should have access to a single, flat price for TB diagnostic test commodities and their related costs. Key TB product procurers, including the Global Drug Facility (GDF), UNDP, and country governments, should work together to negotiate better agreements with diagnostic developers on pricing and access. ***Companies must price products affordably and transparently, with a single low price for all low- and middle-income or high-burden countries, and transparent volume-based milestones established for further price reductions.***

- **Uptake**: though the complexity of the recommended diagnostic algorithms and pricing structures are not ideal, they do not excuse the appallingly low uptake at country level of essential tools. Access to Xpert, liquid culture, line probe assays, and, in high TB/HIV burden settings, LAM testing is vital. Yet country governments have been remiss in their introduction of these potentially life-saving tools. ***National TB programs must rapidly update guidance to ensure best diagnostic practices, and procure and implement products accordingly, including working with HIV and other programs when necessary to ensure access to testing.***

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REFERENCES

1. Ethics guidance for the implementation of the End TB Strategy. Geneva: World Health Organization; 2017.
2. Global tuberculosis report 2016. Geneva: World Health Organization; 2016.
3. Cazabon D, Alsdurf H, Satyanarayana S, et al. Quality of tuberculosis care in high burden countries: the urgent need to address gaps in the care cascade. Int J Infect Dis. 2017 Mar 5. doi: 10.1016/j.ijid.2016.10.016.
4. Proposal for a WHO Model list of essential in vitro diagnostics [Internet]. Geneva: World Health Organization; c2017. http://www.who.int/selection_medicines/committees/expert/21/applications/essential_in-vitro_diagnostics_other/en/.
5. Schroeder L, Guarner J, Elbireer A, Castle P, Amukele T. Time for a model list of essential diagnostics. N Engl J Med. 2016;374(26):2511-2514. doi: 10.1056/nejmp1602825.
6. Open letter re: Urgent need for essential diagnostic list. [Internet]. Geneva: World Health Organization; c2017. http://www.treatmentactiongroup.org/sites/default/files/Essential Diagnostic List_WHO_Final.pdf.
7. WHO meeting report of a technical expert consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. [Internet]. Geneva: World Health Organization; 2017. http://apps.who.int/iris/bitstream/10665/254792/1/WHO-HTM-TB-2017.04-eng.pdf?ua=1.
8. FIND report on accuracy study of the Ultra assay - FIND [Internet]. FIND. 2017: https://www.finddx.org/publication/ultra-report/.
9. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis policy guidance [Internet]. Geneva: World Health Organization; c2016. http://apps.who.int/iris/bitstream/10665/249154/1/9789241511186-eng.pdf.
10. Hofmann-Thiel S, Molodtsov N, Antonenka U, et al. Evaluation of the Abbott RealTime MTB and RealTime MTB INH/RIF assays for direct detection of Mycobacterium tuberculosis complex and resistance markers in respiratory and extrapulmonary specimens. J Clin Microbiol. 2016 Dec;54(12):3022-3027. doi: 10.1128/JCM.01144-16.
11. WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. [Internet]. Geneva: World Health Organization; c2017. http://apps.who.int/iris/bitstream/10665/254792/1/WHO-HTM-TB-2017.04-eng.pdf?ua=1.
12. Boehme C. New Diagnostic Technologies for MDR-TB (Abstract 52). Oral abstract presented at annual Conference on Retroviruses and Opportunistic Infections (CROI); 2017; Seattle, Washington. http://www.croiconference.org/sessions/new-diagnostic-technologies-mdr-tb.
13. Huerga H, Ferlazzo G, Bevilacqua P, et al. Incremental yield of including determine-TB LAM assay in diagnostic algorithms for hospitalized and ambulatory HIV-positive patients in Kenya. PLOS ONE. 2017;12(1):e0170976. doi: 10.1371/journal.pone.0170976.
14. Lawn S, Kerkhoff A, Burton R, et al. Diagnostic accuracy, incremental yield and prognostic value of Determine TB-LAM for routine diagnostic testing for tuberculosis in HIV-infected patients requiring acute hospital admission in South Africa: a prospective cohort. BMC Med. 2017;15(1). doi: 10.1186/s12916-017-0822-8.
15. Suwanpimolkul G, Kawkitinarong K, Manosuthi W, et al. Utility of urine Lipoarabinomannan (LAM) in diagnosing Tuberculosis and predicting mortality with and without HIV: prospective TB cohort from the Thailand Big City TB-research Network. Int J Infect Dis. 2017 Apr 27. pii: S1201-9712(17)30129-7. doi: 10.1016/j.ijid.2017.04.017.
16. GLI model TB diagnostic algorithms. [Internet]. Geneva: World Health Organization; 2017. http://stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf.
17. Boehme C. New Diagnostic Technologies.
18. Ibid.
19. Kocmond W. Letter to chairperson. European AIDS Treatment Group. 2017 March 4. http://www.tbonline.info/media/uploads/documents/cepheid.pdf.
20. Boehme C. New Diagnostic Technologies.
21. Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. Lancet Infect Dis. 2017 Feb;17(2):223-234. doi: 10.1016/S1473-3099(16)30407-8.
22. Calligaro G, Zijenah L, Peter J, et al. Effect of new tuberculosis diagnostic technologies on community-based intensified case finding: a multicentre randomised controlled trial. Lancet Infect Dis. 2017;17(4):441-450. doi: 10.1016/s1473-3099(16)30384-x.
23. Cox H, Dickson-Hall L, Ndjeka N, et al. Delays and loss to follow-up before treatment of drug-resistant tuberculosis following implementation of Xpert MTB/RIF in South Africa: A retrospective cohort study. PLOS Med. 2017;14(2):e1002238. doi: 10.1371/journal.pmed.1002238.

24. Pathmanathan I, Date A, Coggin W, Nkengasong J, Piatek A, et al. Rolling out Xpert MTB/RIF for tuberculosis detection in HIV-positive populations: An opportunity for systems strengthening. Afr J Lab Med. 2017;6(2). doi: 10.4102/ajlm.v6i2.460.
25. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. [Internet]. Geneva: World Health
26. Xpert MTB/RIF implementation manual Technical and operational 'how-to': practical considerations. Geneva: World Health Organization;2014 : http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf.
27. WHO monitoring of Xpert MTB/RIF roll-out [Internet]. Geneva: World Health Organization. c2017: http://www.who.int/tb/areas-of-work/laboratory/mtb-rif-rollout/en/.
28. Mid-term Evaluation of the TBXPERT Project [Internet]. Geneva: Unitaid; 2015. http://www.unitaid.org/assets/Mid-term-evaluation-TBXpert-Innovative-diagnostics-for-multi-drug-resistant-tuberculosis-MDR-TB.pdf
29. Cepheid announces World Health Organization prequalification of Xpert HIV-1 Qualitative test broadens access to critical diagnostic results for infants born with HIV [Internet]. 2016. http://ir.cepheid.com/releasedetail.cfm?releaseid=975834.
30. Boehme C. New diagnostic technologies.
31. Ibid.
32. Ibid.
33. RealTime MTB RIF/NIH Resistance package insert number 51-608276/R3.
34. FluoroType MTB | Fluorescence-based detection of the M. tuberculosis complex from patient specimens [Internet]. Hain-lifescience.de. c2017. http://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/fluorotype-mtb.html.
35. Ibid.
36. Azarschab, Pia (Leitung Vertrieb International, Head of International Sales, Hain Lifescience GMBH, Nehren, Germany). E-mail with: Erica Lessem (Treatment Action Group, New York, NY). 2017 April 4.
37. Boehme C. New Diagnostic Technologies.
38. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance [Internet]. Geneva: World Health Organization. c2017. http://www.who.int/tb/publications/lamp-diagnosis-molecular/en/.
39. Eiken GENOME SITE - The principle of LAMP method. Internet. Loopamp.eiken.co.jp. c2017. http://loopamp.eiken.co.jp/e/lamp/.
40. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis Policy guidance [Internet]. Geneva: World Health Organization; c2016. http://apps.who.int/iris/bitstream/10665/249154/1/9789241511186-eng.pdf.
41. Lawn S, Kerkhoff A, Burton R, Schutz C, Boulle A, Vogt M, Gupta-Wright A, et al. Diagnostic accuracy, incremental yield and prognostic value of Determine TB-LAM for routine diagnostic testing for tuberculosis in HIV-infected patients requiring acute hospital admission in South Africa: a prospective cohort. BMC Med. 2017;15(1). doi: 10.1186/s12916-017-0822-8.
42. Huerga H, Ferlazzo G, Bevilacqua P, Kirubi B, Ardizzoni E, Wanjala S, Sitienei J, Bonnet M. Incremental yield of including determine-TB LAM assay in diagnostic algorithms for hospitalized and Ambulatory HIV-positive patients in Kenya. PLOS ONE. 2017;12(1):e0170976. doi: 10.1371/journal.pone.0170976.
43. Suwanpimolkul G, Kawkitinarong K, Manosuthi W, Sophonphan J, Gatechompol S, et al. Utility of urine Lipoarabinomannan (LAM) in diagnosing Tuberculosis and predicting mortality with and without HIV: prospective TB cohort from the Thailand Big City TB-research Network. Int J Infect Dis. 2017 Apr 27. pii: S1201-9712(17)30129-7. doi: 10.1016/j.ijid.2017.04.017.
44. U.S. Department of State. PEPFAR Country/Regional Operational Plan. Washington, D.C.: U.S. President's Emergency Plan for AIDS Relief; 2017. https://www.pepfar.gov/documents/organization/267162.pdf.
45. GLI model TB diagnostic algorithms. Geneva: World Health Organization; c2017. [Internet]. Geneva: World Health Organization; 2016. http://stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf.
46. Kawasaki M, Echiverri C, Gler MT, et al. Lipoarabinomannan in sputum as a potential biomarker for bacterial load and treatment response in adult pulmonary TB patients. 2016 Colorado Mycobacteria Conference. Fort Collins, US: June 7-10, 2016.
47. Ibid.
48. Geiter, L. Lipoarabinomannan (LAM) as a pharmacodynamic biomarker and potential TB drug development tool. 2017 Critical path to TB drug regimens workshop. Washington, US: March 20–23, 2017.

49. Treatment Action Group (Press Release). 2016 Report on Tuberculosis research funding trends, 2005-2015: no time to lose. 2016 October 25. http://www.treatmentactiongroup.org/tbrd2016.

50. BD, FIND, Stop TB & UNDP collaborate to expand access to critical technology across 85 countries for improved TB diagnosis and drug susceptibility [Internet]. Us3.campaign-archive1.com. 2017: http://us3.campaignarchive1.com/?u=85207b84f0f2d8ddc9bd878de&id=a03d4934fc&e=f92356c696.

51. Ibid.

52. Some of this discordance may be due to poor evidence and limitations of phenotypic standards, rather than a flaw in genotypic testing. Evidence suggests that some genotypic testing, e.g. rpoB mutations for rifampin resistance, are more predictive of outcomes than phenoyptic testing (Van Deun, J Clin Mic, 2013). In April 2017, the WHO held a meeting to review phenotypic standards that will integrate pharmacokinetic/pharmacodynamics and patient outcome data for the first time. Significant changes to some critical concentrations (e.g. for moxifloxacin) are expected.

53. Panels Ion AmpliSeq Designer [Internet]. Ampliseq.com. 2017. https://www.ampliseq.com/panels/search.action?searchFacetValues="Research+Area","Infectious+Disease".

54. Boehme C. New diagnostic technologies.

55. McNerney R, Clark T, Campino S, et al. Removing the bottleneck in whole genome sequencing of Mycobacterium tuberculosis for rapid drug resistance analysis: a call to action. Int J Infect Dis. 2017 March;56:130-135. doi: 10.1016/j.ijid.2016.11.422.

56. Schleusener V, Köser CU, Beckert P, Niemann S, Feuerriegel S. Mycobacterium tuberculosis resistance prediction and lineage classification from genome sequencing: comparison of automated analysis tools. Sci Rep. 2017 Apr 20;7:46327. doi: 10.1038/srep46327.

57. ReSeqTB - [Internet]. Platform.reseqtb.org. 2017. https://platform.reseqtb.org/.

58. Kaniga K, Cirillo D, Hoffner S. A Multilaboratory, Multicountry Study To Determine Bedaquiline MIC Quality Control Ranges for Phenotypic Drug Susceptibility Testing. J Clin Microbiol. 2016 Dec;54(12):2956-2962. doi: 10.1128/jcm.01123-16.

59. Pina RZ, Caleffi-Ferracioli KR, Campanerut-Sá PAZ, et al. Pyrazinamide susceptibility testing in Mycobacterium tuberculosis using the fast resazurin microtiter assay plate. Int J Tuberc Lung Dis. 2016 Jan;20(11):1535–8. doi: 10.5588/ijtld.16.0304.

60. Commercial Serodiagnostic Tests for Diagnosis of Tuberculosis Policy Statement [Internet]. Geneva: World Health Organization; c2011. http://apps.who.int/iris/bitstream/10665/44652/1/9789241502054_eng.pdf.

61. Broger T, Basu Roy R, Filomena A, Greef C, Rimmele S, Havumaki J, Danks D, et al. Diagnostic performance of Tuberculosis-specific IgG antibody profiles in patients with presumptive Tuberculosis from two continents. Clin Infect Dis. 2017 Jan;64(7):947-955. doi: 10.1093/cid/cix023.

62. Chest radiography in tuberculosis detection – summary of current WHO recommendations and guidance on programmatic approaches. [Internet]. Geneva: World Health Organization; c2016: http://apps.who.int/iris/bitstream/10665/252424/1/9789241511506-eng.pdf.

63. Ibid.

64. Kelly-Cirino C, Curry P, Marola J, Helstrom N, Salfinger M. Novel multi-day sputum transport reagent works with routine tuberculosis tests and eliminates need for cold chain: Preliminary study of compatibility with the Xpert MTB/RIF assay. Diagn Microbiol Infecti Dis. 2016;86(3):273-276. doi: 10.1016/j.diagmicrobio.2016.08.013.

65. Maharjan B, Kelly-Cirino CD, Weirich A, Curry PS, Hoffman H, Avsar K, et al. Evaluation of OMNIgene SPUTUM-stabilised sputum for long-term transport and Xpert MTB/RIF testing in Nepal. Int J Tuberc Lung Dis. 2016 Dec1;20(12):1661-1667. doi: 10.5588/ijtld.16.0421.

66. Omar S, Peters R, Ismail N, et al. Field evaluation of a novel preservation medium to transport sputum specimens for molecular detection of Mycobacterium tuberculosis in a rural African setting. Trop Med Int Health. 2016;21(6):776-782. doi: 10.1111/tmi.12701.

67. Tebruegge M, Buonsenso D, Brinkmann F, et al. European shortage of purified protein derivative and its impact on tuberculosis screening practices. Int J Tuberc Lung Dis. 2016;20(10):1293-1299. doi: 10.5588/ijtld.15.0975.

68. Ruhwald M, Aggerbeck H, Gallardo R, et al. Safety and efficacy of the C-Tb skin test to diagnose Mycobacterium tuberculosis infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial. Lancet Respir Med. 2017;5(4):259-268. doi: 10.1016/s2213-2600(16)30436-2.

69. Yi L, Sasaki Y, Nagai H, et al. Evaluation of QuantiFERON-TB Gold Plus for detection of Mycobacterium tuberculosis infection in Japan. Sci Rep. 2016 Jul 29;6:30617. doi: 10.1038/srep30617.

70. Barcellini L, Borroni E, Brown J, et al. First evaluation of QuantiFERON-TB Gold Plus performance in contact screening. Eur Respir J. 2016 Nov;48(5):1411-1419. doi: 10.1183/13993003.00510-2016.

71. Ibid.

72. 2016 Report on Tuberculosis Research Funding Trends, 2005–2015: No Time to Lose [Internet]. New York: Treatment Action Group; c2016: http://www.treatmentactiongroup.org/sites/default/files/TB_FUNDING_2016_WEB.pdf.

73. Ibid.