Diagnosis of opportunistic infections: HIV co-infections - tuberculosis

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Purpose of review
Tuberculosis (TB) incidence has declined \(\sim 1.5\%\) annually since 2000, but continued to affect 10.4 million individuals in 2015, with 1/3 remaining undiagnosed or underreported. The diagnosis of TB among those co-infected with HIV is challenging as TB remains the leading cause of death in such individuals. Accurate and rapid diagnosis of active TB will avert mortality in both adults and children, reduce transmission, and assist in timely decisions for antiretroviral therapy initiation. This review describes advances in diagnosing TB, especially among HIV co-infected individuals, highlights national program’s uptake, and impact on patient care.

Recent findings
The TB diagnostic landscape has been transformed over the last 5 years. Molecular diagnostics such as Xpert MTB/RIF, which simultaneously detects Mycobacterium tuberculosis (MTB) resistance to rifampicin, has revolutionized TB control programs. WHO endorsed the use of Xpert MTB/RIF in 2010 for use in HIV/TB co-infected patients, and later in 2013 for use as the initial diagnostic test for all adults and children with signs and symptoms of pulmonary TB. Line probe assays (LPAs) are recommended for the detection of rifampicin and isoniazid resistance in sputum smear-positive specimens and mycobacterial cultures. A second-line line probe assay has been recommended for the diagnosis of extensively drug-resistant (XDR)-TB Assays such as the urine lateral flow (LF)-lipoarabinomannan (LAM), can be used at the point of care (POC) and have a niche role to supplement the diagnosis of TB in seriously ill HIV-infected, hospitalized patients with low CD4 cell counts of less than 100 cells/\(\mu\)l. Polyvalent platforms such as the m2000 (Abbott Molecular) and GeneXpert (Cepheid) offer potential for integration of HIV and TB testing services. While the Research and Development (R&D) pipeline appears to be rich at first glance, there are actually few leads for true POC tests that would allow for earlier TB diagnosis or rapid, comprehensive drug susceptibility testing, especially when considering the very high attrition rates observed between biomarker discovery and product market entry.

Summary
In this review, we describe diagnostic strategies specifically for HIV and TB co-infected individuals. Molecular diagnostics in particular within the past 5 years have revolutionized and ‘disrupted’ this field. They lend themselves to integration of services with platforms capable of polyvalent testing. Impact on patient care is, however, still debatable. What has been highlighted is the need for health system strengthening and for true POC testing that can be used in active case finding.

Keywords
drug resistance, HIV/TB care, implementation, molecular TB diagnostics, TB control, Xpert MTB/RIF

INTRODUCTION
In 2010, it was true to report that TB curative drugs had not changed in 50 years, TB control programs were weak, treatment regimens were lengthy, and medications toxic. There was limited attention to infection control, inadequate investment in R&D and with the HIV epidemic all served to make Mycobacterium tuberculosis (MTB) disease an increasing global threat [1]. Five years on, we see the global incidence of TB slowly decreasing at around 1.5%
TB remains the leading infectious disease causing mortality especially among HIV co-infected individuals. For several years, rapid and reliable tests such as Xpert MTB/RIF have been available but implementation has been slow in many LMICs and the evidence of its impact on reducing the burden of TB is limited. Greater investment in diagnostic innovations that can allow for early TB diagnosis and universal access to drug susceptibility testing for patients at their first encounter with the health system will be essential to end the TB epidemic. Active case finding strategies have been shown to be cost-effective and need to be coupled with the right diagnostics. Further improvements in assay sensitivity are needed especially for HIV co-infected, as current molecular tests perform suboptimally.}

In sub-Saharan Africa bearing the brunt of dual HIV and TB epidemics. TB [including drug-resistant (DR)-TB] is still the leading cause of death among HIV co-infected individuals. This is evident from autopsy studies, of which a systematic review and meta-analysis of 36 eligible studies [5] reported a pooled prevalence of 39.7% [confidence interval (CI) 32.4–47%] in adults. This varied by world region: 63% in South Asia, 43% in sub-Saharan, and 27% in the Americas. A study in South Africa (SA) from the North West province [6*], which has a high (13%) HIV prevalence, reported a quarter of home deaths had evidence of undiagnosed TB disease, emphasizing the burden of TB in the community and underlining the fatal consequences of delayed TB diagnosis and treatment [7].

Diagnosis of TB is particularly difficult among HIV co-infected individuals who may have atypical, nonspecific clinical presentation, and more often (24–61%) smear-negative disease [8] with less cavitary lesions (due to impairment of granuloma formation [9]), along with higher rates of extrapulmonary TB [EPTB] [10,11*,12]. Sputum-based diagnosis is therefore less sensitive among HIV co-infected patients, resulting in more smear-negative TB disease leading to more empiric treatment among those at greatest risk of disease [13]. This requires caution after the REMEMBER (Reducing Early Mortality and Early Morbidity by Empirc Tuberculosis (TB) Treatment Regimens) trial illustrated that empiric TB therapy did not reduce mortality at 24 weeks compared with isoniazid preventive therapy in adult outpatients with advanced HIV disease initiating antiretroviral therapy (ART) [14*].

The emergence of MDR-TB and XDR-TB further highlights the need for sensitive and timely diagnosis. A meta-analysis performed by Mesfin et al. [15] confirmed the association between MDR-TB and HIV, with the odds of having MDR-TB among HIV-positive cases being 24% higher. However, recent studies show HIV co-infection not to be a direct driver for the emergence and transmission of resistant strains [16*]. As mechanistic mathematical modeling approaches show [17*] the vast majority (up to 80% in underresourced settings) of MDR-TB is due to transmission and not acquisition, a change in dogma regarding DR acquisition versus strain transmission is being called for [18]. It is critical too to understand that MDR-TB strains are as equally transmissible as drug-susceptible [19]. As Van Rie and Warren [18] further highlight, transmission of MDR-TB drives the epidemic in high-burden settings, and the TB epidemic can be contained by implementation of active case finding with rapid TB detection and drug resistance detection (at least...
for rifampicin) for all people with signs and symptoms of TB as the greatest number of MDR-TB cases will be among newly diagnosed TB cases [20*].

TB remains one of the top 10 leading causes of death in children (WHO reports 170,000 deaths in 2015) and of concern is ~57% children diagnosed with and treated for TB are HIV-infected in high-burden countries [21*]. In South Africa it is estimated that children younger than 14 years account for 15–20% total TB burden. Diagnosing childhood TB is challenging as the most appropriate specimen to collect depends on age and clinical presentation. Specimens (especially sputum) are also paucibacillary [22], often of poor quality and quantity. A study of ART programs showed sputum smear microscopy and chest X-ray where available was only used in 86% and 52% of TB diagnoses, respectively [23*]. Although WHO recommends Xpert MTB/RIF testing for children, Xpert MTB/RIF (where available) was only used in 8% and culture in 17% cases [24]. Furthermore, a study in Johannesburg showed 67% sputum collected from children younger than 14 years (median age 24 months) was below the required volume for Xpert MTB/RIF testing [25]. Overall, this highlights the need for strengthening the capacity for diagnosis, proactive screening for TB and MDR-TB in inpatient settings and the community [26]. In addition, HIV co-infected individuals require ART scale up, continuous monitoring, and collaboration between HIV and TB control programs. This will require task shifting [27] and integration of services for adults and children [23*]. Screening pregnant women for HIV and TB may also improve access to care [28], and healthcare providers are encouraged to increase competency in linkage to care and integration, which will also enhance prevention in young infants and children [23*].

The poor sensitivity of smear microscopy (38–69%) in HIV-infected individuals has been well described [29], where the presence of 5000–10,000 bacteria are required for visual detection [30], compared with liquid culture, which remains the gold standard at the lowest limit of detection of MTB of ~10–100 cfu/ml [30,31]. The contrast, however, is a poor (yet affordable and easy to perform in a standard laboratory) test that yields a result in less than 24 h compared with a sensitive test that could take longer than 6 weeks [if drug susceptibility testing (DST) is included] and becomes less clinically relevant, and requires a biosafety laboratory environment and skilled operation. The desired diagnostic needs to be fast, accurate, affordable, and capable of being performed in the household or community, and on a range of specimen types (sputum, urine, stool), simultaneously with HIV diagnosis and monitoring. This is becoming a reality through molecular technology specifically for HIV co-infected individuals [11*]. WHO recommends that the Xpert MTB/RIF assay is used as the initial diagnostic for TB for all adults and children with signs and symptoms of TB and especially where the burden of HIV is high and high rates of DR-TB are suspected. Another desirable component becoming available on several of the molecular instruments is the ability to perform more than one type of test on the same platform. Xpert HIV-1 Qualitative test for early infant diagnosis is now prequalified for use by WHO and provides an opportunity for integration of TB and HIV diagnosis, and extend testing services closer to POC. This would also be the case for the Xpert HIV-1 Quantitative test for HIV viral load monitoring [32], once approved, to improve patient access and impact on the 90/90/90 goals.

WHO ENDORSED DIAGNOSTIC TECHNOLOGIES

Xpert MTB/RIF

The Xpert MTB/RIF (Cepheid) is a cartridge-based molecular test that is fully automated only requiring addition of reagent buffer to liquefy and inactivate any TB bacilli present in a clinical specimen [33–35]. Results are reported within 2 h, and include the detection of rifampicin resistance conferring mutations (RIF). The limit of detection of MTB complex (MTBC) with Xpert MTB/RIF on clinical specimens is 150 cfu/ml [34]. Figure 1 simplistically outlines the overall performance of Xpert MTB/RIF with specific reference to performance among HIV-infected individuals.

In summary, Xpert MTB/RIF performs well on adult respiratory specimens compared with culture reference, but less well in sputum smear-negative specimens. This is similar among childhood specimens, with somewhat increased performance of Xpert MTB/RIF in HIV-infected children. Performance of Xpert MTB/RIF is good on lymph node and other tissue specimens and cerebrospinal fluid, with greater sensitivity in HIV-infected individuals’ lymph node tissue. Xpert MTB/RIF is known to have poorer performance in pleural fluid irrespective of smear status.

The impact of Xpert MTB/RIF on patient care has, to date, been described in 33 studies from 22 countries, including 10 sub-Saharan African countries. Of these, 20 studies (refer to Table 1) discuss the impact of the Xpert MTB/RIF in HIV-infected populations; Xpert MTB/RIF increases detection of TB and dramatically reduces treatment initiation times for DR-TB. The placement of
GeneXpert instruments at treatment facilities and at POC facilities result in shortened treatment initiation times than centralized testing. Changes in empiric treatment practice however varies, and overall impact on mortality has not been shown, and in fact studies undertaken to measure it were underpowered [65*].

The Foundation for Innovative New Diagnostics and collaborating partners is currently undertaking a multicenter study in eight countries of the much anticipated Xpert Ultra (Cepheid) test, which promises to reduce the limit of detection of MTB in sputum to the realm of liquid culture (10–100 cfu/ml) [66]. Much has been learnt from the implementation of Xpert MTB/RIF as summarized in Table 2 and includes [67,68] cost and forecast models [69,70], program interfacing [71], socioeconomic trends [72], and quality assessment [73]. Additional innovations, being reported for the first time for possible TB control (currently, developed for GeneXpert instruments), are remote connectivity [74,75*]. Cepheid’s C360 is a web-based software that remotely connects all instruments and centrally collects result run information. This, together with a laboratory information system allows for central program and laboratory monitoring on test and module performance (potentially in real time), with its further application in TB control [76].

Line probe assays
GenoType MTBDRplus, (Hain LifeSciences) was the first commercial line probe assay (LPA) recommended for use by WHO in 2008 [77]. It remains the most widely studied LPA. Further data have since been published on the use of LPAs and newer versions of LPA technology have since been developed: Hain Genotype MTBDRplus version 2 [78–81]; and the Nipro NTM+MDRTB detection kit developed by
the Nipro Corporation (Japan) DR-TB [82*]. These newer LPAs aim to improve sensitivity for MTBC detection and to simultaneously detect resistance to rifampicin and isoniazid. These LPAs are recommended for use in regional or centralized high-throughput laboratories for the rapid detection of rifampicin and isoniazid resistance in sputum-smear-positive specimens and from mycobacterial cultures. The tests are not recommended for use on sputum smear-negative specimens [83]. LPA require laboratory-trained personnel skilled in PCR, to perform the assay in well managed laboratories. LPA identify drug resistance through manual extraction of MTBC DNA and PCR amplification of the resistance hotspot regions in the rpoB, inhA, and katG genes. The impact of expanded testing using the LPA

<table>
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<th>Main study investigations</th>
<th>Main study findings</th>
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<tr>
<td>Passive TB case detection and treatment outcomes [47]</td>
<td>Increased detection, earlier treatment, decreased empiric treatment and more patients completed treatment with fewer LTFU</td>
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<td>Single Xpert MTB/RIF in intensified case findings among prisoners [48]</td>
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<tr>
<td>Xpert MTB/RIF among smear-negative or sputum-scarce using BAL [37]</td>
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<td>TB management and outcome in hospitalized patients [48]</td>
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<td>DR Screening in a referral hospital [50,51]</td>
<td>Presumptive screening enabled rapid diagnosis (2 days with Xpert MTB/RIF, 8 days to confirm with LPA) and reduced treatment time (8 vs. 40 days) with potential to decrease transmission</td>
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<td>Decentralized care on Rif resistant treatment initiation [52*]</td>
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<tr>
<td>Xpert MTB/RIF impact depends on service coordination [53]</td>
<td>Shortened time to treatment only in TB treatment facilities</td>
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<tr>
<td>Drug-resistant screening in referral hospital [49]</td>
<td>Added little to clinical decision</td>
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<tr>
<td>POC sites [54,55*], intensification case finding [57]</td>
<td>Increased number patients evaluated for DR-TB, time to treat reduced 0–1 days POC vs. 5–11 days vs. centralized Xpert testing</td>
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<td>POC [55+] at PHC [58]</td>
<td>Empirical treatment resulted in no difference in treatment numbers</td>
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<td>Replaces routine [59]</td>
<td>Proportion of patients treated without confirmation was halved</td>
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<tr>
<td>High rates of empirical undermine Xpert MTB/RIF potential [60]</td>
<td>Health-care workers more confident to withhold TB treatment when Xpert MTB/RIF negative and no HIV. No change in morbidity</td>
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<tr>
<td>RCT [61*]</td>
<td>No difference in treatment initiation times, many patients still treated empirically, mortality not reduced</td>
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<tr>
<td>TB management and outcome in hospitalized patients [49] and Intensified case finding [57]</td>
<td>No difference in time to treatment or 2-month mortality</td>
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<tr>
<td>Cluster randomized trial at early country implementation [62*]</td>
<td>No difference in mortality at 6 months</td>
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<tr>
<td>Stepped-wedge RCT [59]</td>
<td>35% reduction in TB related mortality but no increase in treatment success or decrease in LTFU</td>
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<tr>
<td>Single POC Xpert MTB/RIF in PHC [63]</td>
<td>No impact on treatment initiation or mortality among HIV-positive cohort</td>
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<td>Dynamic simulation and economic evaluation [64]</td>
<td>Xpert MTB/RIF could reduce morbidity and mortality, but modestly reduce TB incidence and requires better TB case finding, at substantial program cost</td>
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ART, antiretroviral therapy; LPA, line probe assay; LTFU, lost to follow up; POC, point of care; TB, tuberculosis.
HIV and Diagnostics

Table 2. Considerations for implementation of new diagnostics

<table>
<thead>
<tr>
<th>Build a good team</th>
<th>Trainers (technical, clinical), program managers, data analysts, TB and HIV specialists, R&amp;D scientists</th>
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<tbody>
<tr>
<td>Develop models and trend analyses</td>
<td>Cost, forecast [69,70], program interface [71], socioeconomic trends [72]</td>
</tr>
<tr>
<td>Maintain networks</td>
<td>WHO, new developers, NDOH – stakeholders, funders, clinical lab interface</td>
</tr>
<tr>
<td>Sustain quality management system</td>
<td>EQA [73], connectivity [74,75], manage assay change over, SOPs, infrastructure, HR, stock control, algorithms*</td>
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<tr>
<td>Monitoring and evaluation</td>
<td>Investment case update, epidemiology, and surveillance</td>
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<tr>
<td>Align parallel programs</td>
<td>Correctional facilities, children, EPTB, rural/remote communities through mobile/POC</td>
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<tr>
<td>Integration of services</td>
<td>Platform optimization and service refinement</td>
</tr>
<tr>
<td>Linkage to care</td>
<td>Maximize laboratory information system for faster data streams (SMS printers, m-health)</td>
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Experience drawn from Xpert MTB/RIF implementation [67,68]. EQA, external quality assurance; HR, human resources; NDOH, National Department of Health; POC, point of care; SMS, short message service; SOP, standard operating procedure; TB, tuberculosis.

*Some of the 12 components of the quality assurance model mentioned.

In South Africa resulted in a substantial increase in the proportion of new cases identified as MDR-TB, and although the time to treatment was reduced, it still took 2 months [84].

A second-line line probe assay (SL-LPA) for the detection of resistance to second-line anti-TB drugs – MTBDRsl assay (Hain LifeSciences), incorporates probes to detect mutations within genes (gyrA and rrs) for version 1.0 [85] and, in addition, gyrB and the eis promoter for version 2.0 [86–88], which are associated with resistance to the class of fluoroquinolones or the second-line injectable agents. The presence of mutations in these regions does not necessarily imply resistance to all the drugs within that class. Although specific mutations within these regions may be associated with different levels of resistance (i.e., different minimum inhibitory concentrations) to each drug within these classes, the extent of cross-resistance is not completely understood. WHO recommends the use of SL-LPA as an initial test to detect resistance directly on sputum from patients diagnosed with resistance to rifampicin or MDR-TB [88].

Loop-mediated isothermal amplification assay

Loop-mediated isothermal amplification (LAMP) is a unique, temperature-independent technique for amplifying DNA that is simple to use, providing a visual display that is easy to read. TB-LAMP does not require sophisticated instrumentation and can be used at a peripheral health center level, given biosafety requirements similar to microscopy. A meta-analysis of 10 studies reported a sensitivity of 80% (78–83%) and specificity of 96% (95–97%) to diagnose pulmonary TB [89]. One of the datasets included in the meta-analysis stood out in terms of its findings: the study conducted in Malawi among individuals with cough (44% HIV positivity) reported a sensitivity of 65% (48–79%), specificity of 100% (98–100%), similar performance (P = 0.132) to Xpert, but lower performance compared with concentrated fluorescent smear microscopy with duplicate reading (P = 0.02) [90]. TB-LAMP, however, provides better results than sputum smear microscopy, detecting 15% more patients with pulmonary TB, if performed in all persons presenting with signs and symptoms. If used as an add-on test after microscopy has been performed, more than 40% increase in TB cases were detected among those with smear-negative results compared with other rapid tests that have been recommended by WHO in recent years.

TB-LAMP only detects TB [therefore only suitable for testing of patients at low risk of multidrug-resistant TB (MDR-TB)], and therefore should not replace Xpert MTB/RIF, which simultaneously detects TB and rifampicin resistance. TB-LAMP may be a plausible alternative in settings with low prevalence of HIV and low prevalence of drug resistance, especially where environmental conditions (unstable electricity, temperature, humidity, excessive dust [91]) and possible cost limit access to implementation of Xpert MTB/RIF. The test does not detect drug resistance. It can be performed outside of conventional laboratories but requires training of healthcare staff, similar to the training needed for performing sputum smear microscopy [92].

Lipoarabinomannan assay

LAM is a lateral flow assay requiring 60μl urine and visual reading of band intensity compared with the manufacturer’s supplied reference line on a card to report a result within 25 min, making it applicable to identify active TB at POC, but will require a good quality framework to ensure accuracy. WHO only
TECHNOLOGIES UNDER EVALUATION

Abbott RealTime MTB and MTB-RIF/INH assays

The m2000 (Abbott Molecular, Chicago, Illinois, USA) platform is widely used for centralized HIV viral load testing. The platforms’ flexible, automated extraction and closed real-time PCR systems (testing 93 specimens/8 h day), lend itself to other molecular assays such as the Abbott RealTime MTB (amplification and detection of IS6110 and protein antigen B) and MTB-RIF/INH (similar region detection to those reported by MTBDRplus) assays (Abbott Molecular) for qualitative detection of MTBC [97,98*]. Similar performance to Xpert MTB/RIF in high TB and HIV settings has been noted (Scott LE, unpublished data), and with the added advantage of reporting RIF and INH susceptibility simultaneously [99*]. The m2000 platform has full connectivity functionality, and training and quality management systems are in place in many HIV and TB high-burden countries. Placement for TB testing would be similar to HIV viral load testing, therefore lending itself to integration of HIV and TB laboratory services. This principle of platform integration is not new to molecular testing services and is now also being investigated by Cepheid to provide HIV viral load testing (Xpert HIV-1) on their GeneXpert platform. Therefore, integration of HIV and TB services is not only patient centric now but platform and testing service centric too.

Future

The development pipeline for diagnosing active (including drug resistant) TB appears rich from a molecular diagnostics perspective [100], including a large focus on whole genome sequencing and next-generation sequencing [101,102*]. The aim is to improve sensitivity, speed, ease of use, ability to discriminate TB from other inflammatory or autoimmune diseases and identify subclinical TB in HIV infection [103]. However, very few candidate assays are in the R&D pipeline for true point-of-care tests in rapid diagnostic test format, with disappointing results from biomarker research [104]. There are molecular platforms in the pipeline that will get us closer to patients, but it remains unclear whether test implementation would be cost-effective [105*,106*]. Xpert Omni (Cepheid) may address the criticism of GeneXpert, which requires a laboratory infrastructure (e.g., because of the need for continuous and stable electrical supply) and has limited utility for community testing. The anticipated launch of Xpert Omni in 2017 does not leave much time to address issues for implementers of regulatory assurance, quality control and maintenance, staff resources, logistic support, and cost [107]. Mobile phone and thus platform connectivity may be a particularly challenging field for countries to address. Future evaluation studies will also require broader design to assess impact of TB diagnostics and more attention paid to analyses in methodology studies [108]. This too will apply to evaluation of high throughput centralized testing platforms (e.g., m2000) that will require flexibility around the informed consent process required for trials to match the platforms daily testing throughput.

CONCLUSION

The last few years have seen improvements in the integration of TB and HIV diagnosis and care. New polyvalent platforms should ease integration from a diagnostic standpoint. The major gaps today are true POC testing for early and active case detection and universal rapid DST. Although new tests have transformed TB control and acted as catalyst for change, impact is lower than anticipated. The linkage to care must be optimized to fully capitalize on the potential of new TB diagnostics [109]. Innovation and support is needed not only in the form of new tests, but more importantly for the strengthening of healthcare and delivery services to improve the cascade of care. Only with a comprehensive approach will we be able to achieve the sustainable development goals.

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Conflicts of interest
The authors have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest

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