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Diagnosis of opportunistic infections: HIV co-infections: Tuberculosis

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Abstract

Purpose of the review—TB incidence has declined ~1.5% annually since 2000, but continued to affect 10.4 million individuals in 2015, with 1/3 remaining undiagnosed or under reported. 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 timeous decisions for ART 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 *M. tuberculosis* resistance to rifampicin, has revolutionised 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<100cells/μl. Polyvalent platforms such as the *m2000* (Abbott Molecular, IL, USA)

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and GeneXpert (Cepheid, CA, USA) 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

Molecular TB diagnostics; HIV/TB care; Xpert MTB/RIF; drug resistance; implementation; TB control

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 *M. tuberculosis* (MTB) disease an increasing global threat [1]. Five years on, we see the global incidence of TB slowly decreasing at around 1.5% per annum but still well below the 4–5% annual reduction in incidence needed to meet the first milestones (35% reduction in the number of TB deaths; 20% reduction in TB incidence) of the End TB strategy, set for 2020 [2]. WHO has recommended the use of a standardised shorter multi-drug resistant tuberculosis (MDR)-TB regimen of 9–12 months for the majority of patients (excluding pregnant women) with pulmonary MDR/rifampicin-resistant(RR)-TB that is not resistant to second-line drugs. WHO recommendations for the use of two new drugs, notably bedaquiline and delamanid, have helped to improve outcomes for patients with MDR/XDR-TB. Since 2010, WHO has endorsed the use of several new diagnostic technologies such as Xpert MTB/RIF (Cepheid, CA, USA), molecular line probe assays (Hain Lifesciences, Germany and Nipro Coorporation, Japan) for the diagnosis of MDR- and XDR-TB, TB-LAMP (Eiken, Japan) and LF-LAM (Alere Inc, MA, USA).

In 2016, WHO reports the status of the TB epidemic with 10.4 million new incident cases [3]. TB remains the leading infectious disease causing mortality with an estimated 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among people living with HIV. An estimated 11 % of TB patients are co-infected with HIV and high rates (3.9% of new TB cases and 21% of previously treated cases) of MDR-TB of which 9.5% have XDR-TB represent a major public health crisis [4]. Of note, 80% estimated incident TB cases are reported from 22 high burden countries, with those 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, emphasising 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, non-specific 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 extra-pulmonary TB [10–12]. 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 Trial illustrated that empiric TB therapy did not reduce mortality at 24 weeks compared to isoniazid preventative therapy (IPT) in adult outpatients with advanced HIV disease initiating ART [14].

The emergence of MDR- 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 modelling approaches show [17] the vast majority (up to 80% in under-resourced 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 ten 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 children <14yrs account for 15–20% total TB burden. Diagnosing childhood TB is challenging since 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 programmes showed sputum smear microscopy and chest X-ray (CXR) where available, were only used in 86% and 52% of TB diagnoses [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]. Further, a study in Johannesburg showed 67% sputum collected from children <14yrs (median age 24months) 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 in-patient settings and the community [26]. In addition, HIV co-infected individuals require antiretroviral therapy (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 health care 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 to 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 <24hrs compared to a sensitive test that could take longer than 6 weeks (if 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 >1 type of test on the same platform. Xpert HIV-1 Qualitative test for early infant diagnosis is now pre-qualified 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 (Gous NM, unpublished data), once approved, to improve patient access and impact on the 90/90/90 goals.

WHO endorsed diagnostic technologies

Xpert MTB/RIF (Cepheid)

The Xpert MTB/RIF (Cepheid, CA, USA) 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 [32–34]. Results are reported within 2 hours, 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 150cfu/ml [33]. Figure 1 simplistically outlines the overall performance of Xpert MTB/RIF with specific reference to performance among HIV infected.

In summary, Xpert MTB/RIF performs well on adult respiratory specimens compared to 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 CSF, 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 results in shortened treatment initiation times than centralised 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 [64].

FIND and collaborating partners is currently undertaking a multi-centre study in 8 countries of the much anticipated Xpert Ultra (Cepheid, CA, USA) test, which promises to reduce the limit of detection of MTB in sputum to the realm of liquid culture (10–100cfu/ml) [65]. Much has been learnt from the implementation of Xpert MTB/RIF as summarised in Table 2 and includes [66, 67] cost and forecast models [68, 69], program interfacing [70], socio-economic trends [71], and quality assessment [72]. Additional innovations, being reported for the first time for possible TB control (currently, developed for GeneXpert instruments), is remote connectivity [73, 74]. 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 (LIS) allows for central program and laboratory monitoring on test and module performance (potentially in real time), with its further application in TB control [75].

Line probe assays (LPA)

GenoType® MTBDR*plus,* (Hain Lifescience, Germany) was the first commercial LPA recommended for use by WHO in 2008 [76]. It remains the most widely studied LPA. Further data has since been published on the use of LPAs and newer versions of LPA technology have since been developed: i) Hain Genotype MTBDR*plus* version 2 [77–80]; and ii) the Nipro NTM+MDRTB detection kit developed by the Nipro Corporation (Japan) DR-TB [81]. 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 centralised high through-put 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 [82]. 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 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 [83].

A second-line line probe assay (SL-LPA) for the detection of resistance to second line anti-TB drugs - MTBDR*sl* assay (Hain LifeScience, Germany), incorporates probes to detect mutations within genes (*gyr*A and *rrs)* for version 1.0 [84] and, in addition, *gyr*B and the eis

promoter for version 2.0 [85–87]), 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 RIF or MDR-TB [87].

The loop mediated isothermal amplification assay (TB-LAMP)

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 [88]. One of the data sets 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 to concentrated fluorescent smear microscopy with duplicate reading (p=0.02) [89]. 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, >40% increase in TB cases were detected among those with smear-negative results compared to 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 [90]) 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 health care staff, similar to the training needed for performing sputum smear microscopy [91].

Lipoarabinomannan assay

LAM is a lateral flow assay requiring 60μl urine and visual reading of band intensity compared to the manufacturer's supplied reference line on a card to report a result within 25 minutes, making it applicable to identify active TB at POC, but will require a good quality framework to ensure accuracy. WHO only recommends its use in HIV positive hospitalised individuals, whose CD4 count <100cells/μl [92], and for seriously ill persons irrespective of their CD4 count [93]. A meta-analysis in this population reports the LAM used to diagnose TB with a pooled sensitivity of 56% (41–70) and pooled specificity 90% (81–95). Combining Xpert MTB/RIF testing of urine with urine LF-LAM improved overall TB

diagnostic sensitivity 75% (61–87) and specificity of 93% (81–97) with the added advantage of Xpert MTB/RIF simultaneously detecting susceptibility to RIF [94]. Peter et al showed that POC LAM reduced mortality at 8 weeks in hospitalised patients [95].

Technologies under evaluation

Abbott Real*Time* MTB and MTB-RIF/INH assays (Abbott Molecular, IL, USA)

The *m2000* 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 hour day), lend itself to other molecular assays such as the Abbott RealTi*m*e MTB (amplification and detection of IS6110 and protein antigen B) and MTB-RIF/INH (similar region detection to those reported by MTBDR*plus*) assays for qualitative detection of MTBC [96, 97]. 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 [98]. 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) tuberculosis appears rich from a molecular diagnostics perspective [99], including a large focus on whole genome sequencing and next generation sequencing [100, 101]. 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 [102]. However, very few candidate assays are in the R&D pipeline for true point-of-care tests in RDT format, with disappointing results from biomarker research [103]. 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 [104, 105]. Xpert Omni (Cepheid, CA, USA) 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 [106]. 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 [107]. This too will apply to evaluation of high throughput centralised 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 POCT for early and active case detection and universal rapid DST. While 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 [108]. Innovation and support is needed not only in the form of new tests, but more importantly for the strengthening of health care 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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Key points

- 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.

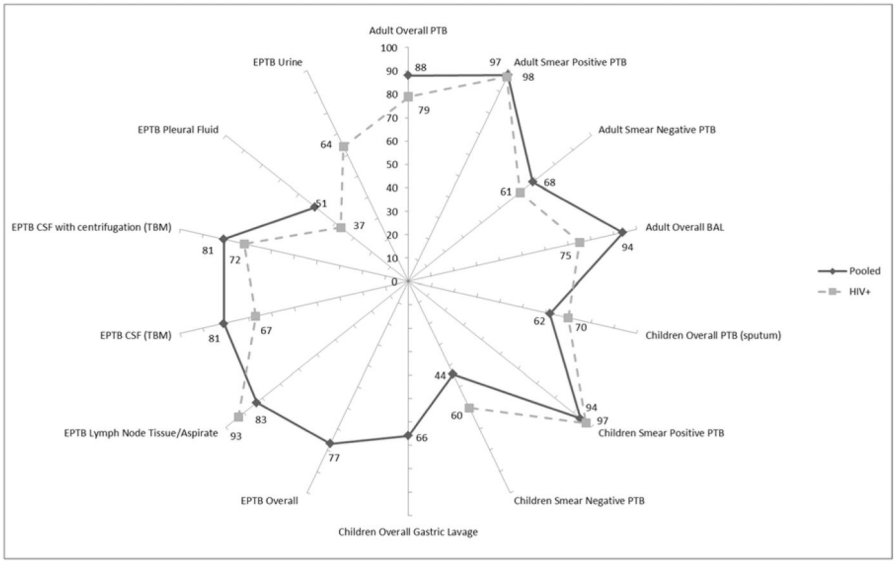

Figure 1.

A radar plot of the performance (represented as sensitivity values 0–100%) of Xpert MTB/RIF compared to liquid culture for various specimen types. PTB-pulmonary tuberculosis, BAL-bronchoalveolar lavage, EPTB, CSF-cerebrospinal fluid and TBM-tuberculosis meningitis. The solid radial arms illustrate sensitivity (%) of overall pooled data, and the dashed radial arms report studies among HIV/TB co-infected individuals. Adults Overall: n=27 studies (7 by HIV status), Smear Positive PTB: n=5 studies, Smear Negative PTB: n=5 studies [35]; BAL: n=1 study, centrifuged BAL [36]. Children Overall PTB (sputum): n=12 studies, Smear Positive PTB: n=6 studies, Smear Negative PTB: n=7 studies, Overall Gastric Lavage: n=7 studies [37]. EPTB Overall EPTB: n=36 studies [38]; Lymph Node Tissue/Aspirate: n=18 studies [39], n= 1 HIV positive cohort [40]; CFS(TBM): n=18 studies [39], n=1 HIV positive cohort, mixed CSF samples [41], n=1 HIV positive cohort, centrifuged CSF [42]; Pleural fluid: n=24 studies [43], n=1 high TB-HIV prevalence cohort [44]; Urine: n=1 hospitalised, HIV positive cohort [45].

Table 1

Impact of Xpert MTB/RIF specifically among HIV co-infected individuals

	Main study investigations	Main study findings
Detection of TB	Passive TB case detection and treatment outcomes [46]	Increased detection, earlier treatment, decreased empiric treatment and more patients completed treatment with fewer LTFU
	Single Xpert MTB/RIF in intensified case findings among prisoners [47]	
	Xpert MTB/RIF among smear-negative or sputum-scarce using BAL [36]	
	TB management and outcome in hospitalized patients [48]	
Drug resistant (DR)-TB	DR Screening in a referral hospital [49, 50]	Presumptive screening enabled rapid diagnosis (2days with Xpert MTB/RIF, 8 days to confirm with LPA) and reduced treatment time (8days vs 40days) with potential to decrease transmission
	Decentralised care on Rif Resistant treatment initiation [51]	
Placement (services)	Xpert MTB/RIF impact depends on service coordination [52]	Shortened time to treatment only in TB treatment facilities,
	Drug resistant screening in referral hospital [49]	Added little to clinical decision
	POC sites [53–55] and intensified case finding [56]	Increased number patients evaluated for DR-TB, time to treat reduced 0 – 1 days POC vs 5–11 days vs centralised Xpert testing
Empiric treatment	POC [54]	Empiric treatment resulted in no difference in treatment numbers
	at PHC [57]	Proportion of patients treated without confirmation was halved
	Replaces routine [58]	Health-care workers more confident to withhold TB treatment when Xpert MTB/RIF negative and no HIV.
	High rates of empiric undermine Xpert MTB/RIF potential [59]	No change in morbidity,
ART associated TB	RCT [60]	No difference in treatment initiation times, many patients still treated empirically, mortality not reduced
Mortality	TB management and outcome in hospitalized patients [48] and Intensified case finding [56]	No difference in time to treatment or 2month mortality
	Cluster randomized trial at early country implementation [61]	No difference in mortality at 6 months
	Stepped-wedge RCT [58]	35% reduction in TB related mortality but no increase in treatment success or decrease in LTFU
	Single POC Xpert MTB/RIF in PHC [62]	No impact on treatment initiation or mortality among HIV positive cohort
	Dynamic simulation and economic evaluation [63]	Xpert MTB/RIF could reduce morbidity and mortality, but modestly reduce TB incidence and requires better TB case finding, but at substantial program cost

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Table 2

Considerations for implementation of new diagnostics *(experience drawn from Xpert MTB/RIF implementation* [66, 67])

Build a good team	Trainers (technical, clinical), program managers, data analysts, TB and HIV specialists, R&D scientists
Develop models and trend analyses	Cost, forecast [68, 69], program interface [70], socioeconomic trends [71]
Maintain networks	WHO, new developers, NDOH – stakeholders, funders, clinical lab interface
Sustain quality management system	*EQA [72], Connectivity [73–75], manage assay change over, SOPs, infrastructure, HR, stock control, algorithms
Monitoring and evaluation	Investment case update, epidemiology and surveillance
Align Parallel programs	Correctional facilities, children, EPTB, rural/remote communities through mobile/POC
Integration of services	Platform optimization and service refinement
Linkage to care	Maximize laboratory LIS for faster data streams (SMS printers, m-health)

*some of the 12 components of the quality assurance model mentioned