



PULMONOLOGY

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REVIEW

Point of care diagnostics for tuberculosis

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Received 27 November 2017; accepted 7 December 2017

KEYWORDS

Tuberculosis;
Point-of-care;
Diagnosis;
Target product
profile;
Xpert;
LAM;
NAATs

Abstract The goals of the End TB strategy, which aims to achieve a 90% reduction in tuberculosis (TB) incidence and a 95% reduction in TB mortality by 2035, will not be achieved without new tools to fight TB. These include improved point of care (POC) diagnostic tests that are meant to be delivered at the most decentralised levels of care where the patients make the initial contact with the health system, as well as within the community. These tests should be able to be performed on an easily accessible sample and provide results in a timely manner, allowing a quick treatment turnaround time of a few minutes or hours (in a single clinical encounter), hence avoiding patient loss-to-follow-up. There have been exciting developments in recent years, including the WHO endorsement of Xpert MTB/RIF, Xpert MTB/RIF Ultra, loop-mediated isothermal amplification (TB-LAMP) and lateral flow lipoarabinomannan (LAM). However, these tests have limitations that must be overcome before they can be optimally applied at the POC.

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<https://doi.org/10.1016/j.rppnen.2017.12.002>

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Furthermore, worrying short- to medium-term gaps exist in the POC diagnostic test development pipeline. Thus, not only is better implementation of existing tools and algorithms needed, but new research is required to develop new POC tests that allow the TB community to truly make an impact and find the ‘‘missed TB cases’’.

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Introduction

Tuberculosis (TB) remains the leading infectious cause of death worldwide. The World Health Organization (WHO) estimated around 10.4 million new cases of TB in 2016 but less than two-thirds of these were diagnosed or reported to health authorities.¹ The ambitious goal of the End TB strategy which aims to achieve 90% reduction in incidence and 95% reduction in mortality by 2035² will not be possible without new tools to fight TB (more effective vaccines, shorter treatment regimens, and improved diagnostic tests). Proper and rapid diagnosis is key to control TB.

‘‘Without diagnosis, medicine is blind’’³ and all other efforts directed to provide adequate and prompt treatment, and hence reduce transmission, can not be undertaken without diagnosis. Improved testing means not only developing highly sensitive and specific assays to diagnose TB and drug resistance but also tests that are affordable, rapid, and have the capacity to be deployed at the most decentralised level (point of care, POC) by health care workers with minimal training. Importantly, it is critical for programmes to view the diagnostic process holistically: for example, POC diagnosis can be improved by strengthening infrastructure at primary care, which is poor in most high burden countries (e.g., through the provision of stable electricity), without necessarily having a new testing technology.⁴

Nonetheless, in the last decade we have witnessed formidable progress in the field of TB diagnostics. Several assays, such as Xpert MTB/RIF (Xpert), Xpert MTB/RIF Ultra (Ultra), urine lateral flow lipoarabinomannan (LF-LAM) or loop-mediated isothermal amplification (TB-LAMP) have been WHO endorsed and are being rolled out progressively.^{5–8} These ‘‘approved’’ tests are meant to be used at different levels of care and with different advantages and limitations (Fig. 1).

This review aims to provide a snapshot of current assays thought to be the most useful at POC. Many of these tests do not meet the ideal characteristics of a POC test but may still be useful. We will also discuss novel future assays with POC potential.

Point of care vs centralised testing

Although improved tests doable at health facilities with basic laboratory infrastructure are needed, there a higher urgency for tests deployable at rural TB facilities at community level. Detecting cases in these decentralised settings, often coinciding with areas with poorer health care access, is critical.

Most patients do not start treatment the day of specimen provision. There is a reasonable consensus among the research community that TB-POC tests must be deployable at the most decentralised levels of care where the patients make the initial contact with the health system, as well as in the community itself. In addition, POC tests need to lead to a rapid change in patient management (if appropriate).⁹ Thus, a POC should be able to be performed in an easily accessible sample and provide results in a timely manner, allowing treatment times of hours and hence avoiding patient loss-to-follow-up. We therefore considered only tests with the potential to meet these criteria, either in rural settings or well-resourced urban clinics.

Point of care test target product profile

The WHO released a series of high priority target product profiles (TPPs) for TB diagnosis at POC: (1) a non-sputum-based test capable of detecting all forms of TB by identifying characteristic biomarkers or biosignatures, (2) a triage test that can be used by first-contact health-care providers to identify those who need further testing, (3) a sputum-based test to replace smear microscopy for detecting pulmonary TB.¹⁰

The POC biomarker test (for non sputum samples) should enable the diagnosis of both pulmonary and extra pulmonary tuberculosis, paediatric TB or at early stages of the disease. It would need to be at least as sensitive as other POC tests in sputum (Xpert), portable and. The triage test would be applied to high risk patients, most of whom would not have TB and would have minimal symptoms. Thus, the test needs to be simple, low cost and highly sensitive. It is likely that this triage test would be performed in the simplest available sample, such as breath, blood or urine. The sputum replacement POC assay needs to be at least as sensitive as Xpert, robust and with a fast turnaround time without the drawbacks of Xpert (i.e., able to be used without power needs or temperature control). It is unfeasible, at least in the short term, that a single test can include all these characteristics (Table 1).

Current point-of-care or near-point-of-care tests

Smear microscopy

Smear microscopy consists of examining specimen under a microscope to detect acid fast bacilli after staining with Ziehl–Neelsen or a Auramine.¹¹

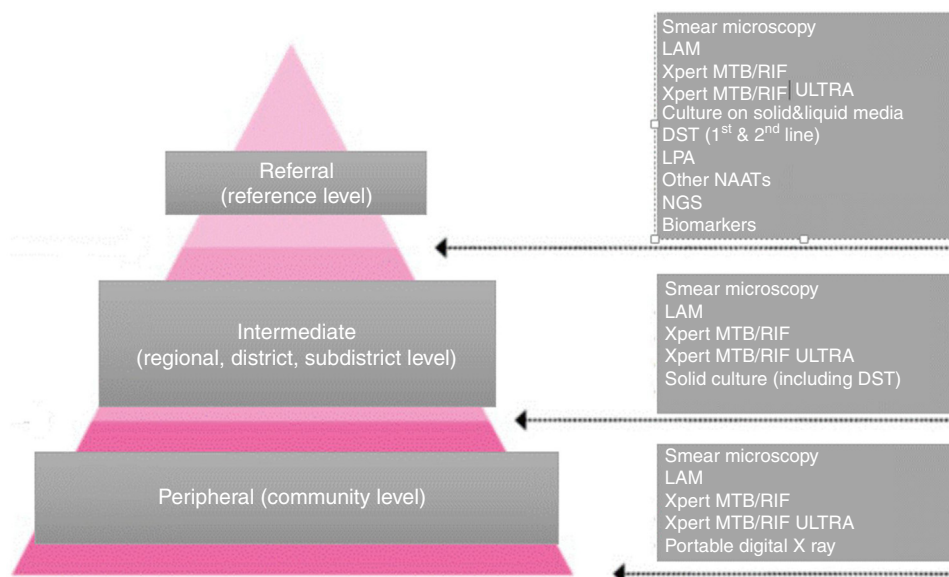


Figure 1 The three tiers of the network of TB laboratories and the responsibilities and the tests offered at each level. Adapted from WHO.¹⁵⁶

Table 1 TPP of an ideal POC test for diagnosis tuberculosis.¹⁰

Ideal characteristics of a POC assay for diagnosing Tuberculosis

- It can detect pulmonary or extrapulmonary tuberculosis (TB)
- It can be used in children and adults, HIV-positive or HIV-negative TB presumptive cases
- It can be used in several easily accessible body samples
- Used by health care workers with minimal training
- Used in peripheral health facilities or community
- It can be operated in broad ranges of temperature and humidity
- Delivers results in less than 20 min
- No or minimal maintenance required
- Cheap (less than 4 US dollars per test)
- High sensitivity
Sensitivity similar to that of Xpert MTB/RIF for pulmonary and extrapulmonary TB
If intended to use as a triage test (95% compared to culture)
- High specificity
Specificity similar to Xpert MTB/RIF for pulmonary and extrapulmonary TB
If intended to be use as a triage test (80% compared to culture)

Sputum smear microscopy is one of the most effective tools for identifying people with infectious tuberculosis. Smear-positive patients are up to 10 times more infectious than smear-negative patients.¹² The threshold of detection of AFB in sputum is 10^4 – 10^5 CFU/ml. It is still the primary method for diagnosis of TB in low and middle income countries (LMIC).^{11,13} In LMIC is the only cost-effective tool for diagnosing infectious patients, monitor their progress in treatment¹⁴ and confirm cure.

Technically, smear microscopy is inexpensive, easy to perform and highly specific in areas with high prevalence. However, sensitivity values are low. Maximum sensitivity has been found to be up to 60% under optimal conditions when compared with that of cultures.¹⁵

Compared to bright-field and fluorescence microscopy, the Ziehl–Neelsen technique is easier to learn. In contrast,

light emitting diode (LED) microscopy is 10% more sensitive than conventional microscopy and 98% specific¹¹ and is considerably cheaper.¹⁶ In 2011, WHO released a new policy on LED Fluorescent Microscopy for TB.¹⁷

Despite microscopy’s advantages of being cheap and rapid, results are, however, seldom on the same day.

Xpert MTB/RIF

Xpert is a real-time quantitative PCR assay for *Mycobacterium tuberculosis*-complex DNA.¹⁸ It amplifies part of the *rpoB* gene that contains mutations that cause rifampicin-resistance (a critical first-line drug). Hence Xpert can detect TB and resistance simultaneously. Xpert has shown high sensitivity and specificity to diagnose pulmonary tuberculosis

Table 2 Xpert MTB/RIF sensitivity and specificity for pulmonary or extrapulmonary tuberculosis.^{19,20}

	Pulmonary tuberculosis		Extra pulmonary tuberculosis	
	Sensitivity (% , 95% CI)	(%, 95% CI)	Sensitivity (% , 95% CI)	Specificity (% , 95% CI)
Overall	89 (84–92)	99 (98–99)	88 (77–95)	98 (87–99)
Smear-negative	68 (61–74)	99 (98–99)	69 (60–80)	
Smear-positive	98 (97–99)		95 (91–100)	
HIV negative	86 (76–92)	99 (98–100)		
HIV positive	79 (70–86)	98 (96–99)		
Smear negative	61 (40–81)			
Smear positive	97 (90–99)			

and extrapulmonary tuberculosis (Table 2).^{19–21} Introduced in 2011, Xpert is WHO-approved as a frontline test for pulmonary, extrapulmonary, and paediatric TB,⁶ undergone widespread global scale-up (~25 million cartridges and 25,000 modules procured by end of 2016),²² revolutionised the diagnosis of TB (75% of smear-negative pulmonary TB cases can now be bacteriologically-detected within 2 h), and paved the way for universal drug susceptibility testing.^{19,23}

Studies have demonstrated Xpert to have POC feasibility in well-resourced clinics (largely due to its semi-automated nature that requires minimally trained non-technical personnel^{24–27}). However, the test has, due to its need for uninterrupted power, mild ambient conditions, and economies of scale (i.e., the expensive instrumentation is most cost-effective when used at volumes that exceeds rates of specimen collection at most individual primary care facilities),²⁸ predominantly been implemented in centralised laboratories.²⁹

The impact of the most widespread, rapid and accurate test for TB that we have (Xpert) has thus potentially been undermined by its far-patient placement. For example, studies of POC vs centralised Xpert have shown POC placement to lead to more cases initiating treatment while maintaining similar rates of diagnostic accuracy.^{30,31} Thus, it appears Xpert's ability to provide POC same-day diagnoses reduces pre-treatment loss-to-follow-up.

Looking beyond Xpert, it is important to ask how can test developers and researchers improve the impact of future POC tests? How can promising research and development activity be capitalised upon, whilst being cognisant of the resource limitations at primary care in high burden settings? What are the "lessons learnt" from Xpert?

In high burden, high HIV settings, the evidence unfortunately suggests that Xpert's impact is small, with no evidence of long-term improvements in patient outcomes like morbidity and mortality.^{24,32,33} This is due to high rates of existing empirical TB treatment (most patients newly-detected by Xpert may have been treated on empirical grounds anyway)³⁴ and the still suboptimal sensitivity in special populations (HIV-positives, children, extrapulmonary TB).^{35–38} Another important factors are weak health systems, which mean that clinics are not able to rapidly start Xpert-positive patients on treatment (this is especially acute for rifampicin-resistant patients³⁹), the limited affordability of Xpert,⁴⁰ and challenges with weak testing infrastructure at POC.^{41,42} Thus, when implementing successor

technologies to Xpert at POC, health providers need to adopt an holistic implementation approach that includes broad systems strengthening.

Interestingly, Xpert has two additional limitations that have dampened enthusiasm in some settings, even when results are generated near POC. These limit Xpert's utility, as they increase reliance on downstream tests like culture. For example, patients with previous TB, who are an epidemiologically-important subpopulation who re-present with symptoms, have old mycobacterial genomic DNA in their lungs that can cause false-positive results^{43,44} (for active TB). Thus, as diagnostic tests improve in sensitivity, specificity is likely to be compromised in patients with a history of TB unless special precautions are taken. Furthermore, in the event of rifampicin-resistance detection, many countries still require confirmatory drug susceptibility testing,⁴⁵ which often requires additional specimen collection, although innovative new approaches of "hacking" the used Xpert cartridge may be useful.⁴⁶

Xpert MTB/RIF Ultra

Ultra is a successor technology to Xpert that uses the same test hardware. Ultra has a limit of detection of 16 CFU/ml (compared to Xpert's 114 CFU/ml Xpert), and uses the same semi-quantitative categories as Xpert (high, medium, low, very low) and a new "trace" that corresponds to the lowest bacillary burden.⁸ If MTB is detected, category "trace", then no interpretation can be made regarding rifampicin resistance and results are reported as 'MTB detected, trace, RIF indeterminate'.⁴⁷

Overall, sensitivity of the Xpert Ultra is 5% higher than that of Xpert (95% CI +2.7, +7.8) but specificity is 3.2% lower (−2.1, −4.7). A higher incremental sensitivity is seen among paucibacillary forms of TB disease (childhood TB, HIV-associated TB, or extrapulmonary TB (1–3)). However, specificity is lower in patients with a history of TB, which means that among these patients, Ultra results should be interpreted carefully, together with a comprehensive clinical history and physical examination.^{8,48}

Since the end of March 2017, the WHO has recommended the replacement of Xpert by Ultra. The current WHO recommendations for the use of Xpert also apply to the use of Xpert Ultra.⁸

GeneXpert OMNI

Given the concerns associated with the use of continuous power and need for a lab with the traditional GeneXpert platform, Cepheid has developed a POC platform. GeneXpert Omni is a single standalone and handheld module that is capable of processing Xpert cartridges in more extreme settings (elevated temperatures and humidity) and has four hours of battery life. It is intended to allow GeneXpert implementation away from central or peripheral settings. Omni is small and portable, weighing only 1.0 kg. It has a supplemental battery that gives an additional 12 h Battery life. As Omni module can do a single assay every ~110 min, cost and accessibility will limit adoption in high-endemic areas. The projected release of the Omni in emerging markets is at the end of 2018, and it has not yet been endorsed by WHO²² nor is there any available evidence to support its use.

Lateral flow lipoarabinomannan commercial tests (LF-LAM)

Lipoarabinomannan glycolipid is a component of the mycobacterial cell wall. In 2001 a proof of concept for an ELISA detection in urine was published⁴⁹ and years later a lateral flow assay for LAM detection in urine was marketed^{50,51} ('Determine TB-LAM', *Alere*, USA). This rapid test can be performed at the bedside, gives a result in 25 minutes and costs <3 USD.

In 2015, the WHO released policy guidance⁷ on LF-LAM assays, stating that the test may be used to 'assist the diagnosis of TB in HIV positive TB presumptive patients who have a CD4 count less or equal to 100 cells/ μ L, or who are seriously ill (present with any one of four 'danger signs')'. It may also be useful in children.^{52,53}

LF-LAM has a pooled sensitivity of 44%, however, this varies significantly according to health settings (54% in hospitalized patients and 21% in outpatients) and CD4 count (15%, 48% and 56% with >200, <200 and <100 cells/ μ L). WHO experts discouraged its use as a screening tool (among HIV-positive patients regardless of symptoms) because of a suboptimal specificity of 92%. This low specificity was contentious because 'false positive' LAM results could have been signalling true TB cases but sputum negative.⁵⁴ In fact, studies that sampled more bodily compartments showed LF-LAM has a specificity of 99%.^{55,56}

LAM positivity shows a strong direct correlation with severity^{57,58} and mortality.⁵⁶⁻⁶⁴ The test would target precisely HIV-infected patients at risk of longer diagnostic delays (sputum negative⁶⁵⁻⁶⁸ or unable to expectorate⁶⁹) and those highly immunocompromised.^{62,64} In 2016 a randomised clinical trial in 10 hospitals of 4 African countries showed a 17% relative risk reduction 8 week mortality regardless of CD4 when treatment initiation was guided by the addition of LF-LAM to standard diagnostics.⁷⁰ Thus, LF-LAM is the only TB diagnostic test with evidence of a mortality benefit.

To better understand LF-LAM's accuracy and mortality impact, it is important to note that evidence suggests that LAM in urine mostly reflects the presence of viable mycobacteria in the kidneys,^{71,72} and blood dissemination. LF-LAM positivity correlates very strongly with results

from autopsies showing renal or disseminated TB,⁷¹ positive mycobacterial blood culture⁷³ and positivity Xpert in concentrated urine.⁶⁹

A study in South Africa showed that clinicians exerting a very low threshold for initiating empiric TB treatment missed cases that would otherwise have been diagnosed if LAM had helped their decisions.⁷⁴ WHO classic algorithms for HIV sputum negative TB suspects and seriously ill adults benefit from including LAM.^{59,74}

Nonetheless, the HIV/TB research community continues to show that LAM in HIV-positive is useful in patients beyond those described in the WHO endorsement^{56,58,62,68,73-78} (or even as a prognostic^{52,64} or treatment response monitoring tool⁶³). This may be due to the increasing availability of this true POC test (rapid, patient-side, low cost), a better understanding of its meaning, and the efforts driven by earlier/rapid antiretroviral treatment initiation for HIV, which can only be safe if rapid TB diagnosis is possible. Nonetheless, despite being the only new TB test with evidence for a mortality benefit, there has not been a large scale adoption of LAM. Further action and advocacy is needed to include this test within TB diagnostic screening algorithms of severely ill HIV patients.

Portable digital chest X-ray (CXR)

CXR is unquestionably a very sensitive test. A recent prevalence survey in Kenya showed 92% sensitivity for TB in HIV-positive and 100% in HIV negative, but also low specificity (73%) with variations depending on the criteria used to consider "positive" certain abnormalities.⁷⁹ CXR is especially useful for screening high-risk populations, for general populations in prevalence surveys, or diagnosis of smear negative TB (HIV positive TB cases or paediatric TB). The test is more sensitive (and no less specific) than asking for symptoms, except when we consider 'any symptom', in which case the sensitivity is similar. When used in decision trees or algorithms, CXR should be placed at the start or in the early stages, rather than at the end (confirmatory).⁸⁰ Unfortunately, the latter is general practice, but only because of operational and logistical constraints that limit its availability to central facilities.

This is changing today. There is recognition that CXR is a good tool in active case finding strategies, filling the gap that symptom-based screening (SBS) leaves: it finds cases that the latter misses (specially among HIV-positive and other vulnerable populations). Combining both, and adding a rapid molecular test for laboratory confirmation of the disease is an ideal and cost-effective strategy (CXR serving as a triage to reduce the number of Xpert tests).⁸¹ The development of digital and portable X-ray systems and, importantly, automated software that obviates the need for an experienced reader, allows to CXR to be considered POC test.⁸² A simple van can carry an X-ray device and GeneXpert to any location.

Mounting evidence shows the success of these so-called mobile units in three scenarios (Fig. 1):

- (1) Detecting TB in hard to reach risk populations in low incidence/high income countries.^{83,84} A recent systematic review⁸⁵ showed this is a highly cost-effective strategy.

- (2) High incidence/medium income countries such as India,^{86,87} Myanmar⁸⁸ or Philippines⁸⁹ are increasingly using mobile clinics to bring GeneXpert and Xray to peripheral/rural facilities where TB suspects are periodically appointed for a one-stop (same day) diagnosis.⁸⁷
- (3) Active case finding campaigns in aggregated settings (prisons, hospices).^{89,90}

The digitalization not only allows portability but also tele-medicine⁹¹ and computer aided diagnosis (CAD). Their specificity, which increases by adding clinical information,⁹² is already coming close to human interpretation⁹³ (except by experienced radiologists)⁸¹ making them a very attractive option in low-resourced settings and remote areas.⁹⁴

Novel/future assays with potential for POC for TB diagnosis

NAATs and next generation sequencing for TB diagnosis (and detection of drug resistance)

Despite the recent advances in automated PCR technology,⁹⁵ what are the next PCR/NAAT technological enhancements necessary for a same-day point of care test? The combination of decentralised health infrastructure and new technology improved ATT initiation from 64 to 18 days.⁹⁶ There are numerous other potential improvements that could give clinicians the ability to diagnose TB and drug resistance on the same day as seeing the patient.

In addition to Xpert, there are other real-time PCR assays including, but not limited to, Artus MTB (Qiagen, Germany), Molbio (Bangalore, India), Anyplex II MTB/MDR (Seegene, Korea) and Abbott RealTime MTB. While these assays are less automated than Xpert, the isolated DNA can be paired with other assays, such as paper-based drug detection via line probe assays. The Abbott assay added additional *Mtb* gene targets to improve sensitivity.⁹⁷ Most of these assays have a relatively small amount of evidence to support their use, require a thermocycler as well DNA isolation and therefore are appropriate in central and some peripheral laboratory settings. To push PCR towards peripheral and community-based testing separate advances are necessary.

Potential future POC diagnostic PCR/advances could come from (1) isothermal technology; (2) paper-based innovations; (3) quantitative DNA ± RNA assays; and (4) improvements in DNA isolation from non-invasive, non-sputum specimens such as urine, stool or dry blood spot. Loop-mediated isothermal amplification (LAMP) has been used extensively, with multiple meta-analysis describing sensitivity and specificity of ~89–93% and 94–95% respectively.^{98–100} In contrast to RT-PCR using a forward and reverse primer, LAMP uses four to 6 primers, does not require an expensive thermocycler and has similar limits of detection (LOD). As it does not need thermocycler or fluorescence detection, LAMP technology has the potential to decrease test cost and time to diagnosis in peripheral labs or community settings.

Similarly, a paper-based assay like the lateral flow assays (LFAs) used for at home pregnancy and HIV testing shows promise for improving TB diagnostics. Proof of concept for nucleic acid LFAs have been successful detecting *Mtb*

at 10⁴ copies of genomic DNA as well as *katG* and *rpoB* genotypic resistance.¹⁰¹ The EasyNAT (Ustar, China) incorporates a syringe-based minimally laborious DNA extraction step with isothermal amplification (no expensive thermocycler required) followed by a simple Lateral flow detection method (LF).¹⁰² While advances in portability, simplicity and eliminating the need for a thermocycler, improvements in limit of detection (LOD), currently >10⁴ CFU/ml, will be needed for ideal sensitivity.¹⁰³

The majority of PCR/s are designed for evaluating sputum specimens, however large proportions of community and peripheral health centres are not capacitated to induce sputum and certain populations (PLHIV, infants, elderly) are unable to expectorate. Modifications of existing tests to use minimally-invasive specimen types such as urine, stool or string test offer the promise of increasing diagnostic yield, genotypic drug resistance detection and treatment monitoring in patient populations more likely to suffer poor outcomes.^{104–108}

NAATs and next generation sequencing for detection of drug resistance

In combating the rising number of cases of drug resistant TB, the ability of Xpert to quickly detect *rpoB* genotypic resistance is improving time to starting appropriate therapy. In order to prescribe the recently WHO-recommended shorter regimen, clinicians should be sure that RR/MDR-TB patients are not resistant to these drugs, thus samples need to be referred to an intermediate level laboratory for rapid testing.^{109–116} As the open and manual format of LPAs and the technical requirements of culture, preclude their use as POC tests,¹¹⁷ it is critical that POC clinics have rapid referral mechanisms in place for specimens for further DST. The advent of the Xpert XDR cartridge might help further DST to be done more widely.¹⁰⁹

Next-generation sequencing (NGS) is the next advance in rapid detection of genotypic resistance with initial studies suggesting the ability to identify resistance earlier and potentially with improved cost effectiveness compared to phenotypic drug susceptibility testing (DST). Preliminary NGS studies detect genotypic resistance with 97–100% concordance compared phenotypic DST.^{118–120} Similar to the line probe assays, it is quite feasible for NGS to be performed directly on smear-positive clinical sputum samples^{119–121} with INH and RIF resistance sensitivities and specificities of 95–97% and 98–100%, respectively. Compared to phenotypic DST, the *potential* improvements in times to drug resistance detection and cost provide cautious optimism.

To become a point of care, or even near point of care in very well-resourced setting, sequencers will need to be portable (the MinION is an early example of this) and complex bioinformatic algorithms will continue to advance in efficacy and timeliness. When implementing NGS directly from sputum, urine or stool, improvements in bacillary enrichment will decrease background noise from other microbes present in sputum and non-sputum based samples. Additional potential benefits from NGS include enhanced contact tracing, detection of subpopulations (heteroresistance) and identification of pathogen virulence factors

(further in-depth reviews of the hopes and hindrances of NGS for TB diagnostics are available).¹²²⁻¹²⁴

Biomarkers (host and pathogen)

A biomarker is a substance, structure or process that can predict the incidence or outcome of a disease.¹²⁵ To be used at POC, biomarkers need to perform in settings with limited laboratory facilities and ideally be low cost and easy to use.¹²⁶ In the last decade, multiple biomarkers with diagnostic potential which could be developed as POC assays in the future¹²⁷ have been identified. However very few are currently available. A recent review of 399 non-DNA potential biomarkers suitable for the POC concluded that only 12 were validated prospectively and only one had reached WHO endorsement for patients with advanced HIV (lipoarabinomannan, LAM, already discussed).¹²⁸ Here we describe examples of some of these other biomarkers with potential use as POC tests (Table 3):

Mtb Ag85: The Mtb Ag85 complex is a family of three proteins (Ag85A, Ag85B, and Ag85C) with enzymatic mycolyl transferase activity involved in the coupling of mycolic acids to the arabinogalactan of the cell wall and in the biogenesis of the cord factor. Although initially promising, its detection in blood and urine shows highly variable performance^{129,130} and is not validated as a POC assay.

Volatile organic compounds (VOCs): Exhaled breath may contain VOCs that could be derived directly from Mtb and/or the host (e.g., products of oxidative stress). The detection of VOCs is difficult because they are excreted in picomolar concentrations and no devices are available for the POC. Several e-nose prototypes (i.e., eNosi Aeonose), point-of-care electronic nose device to diagnose TB through exhaled breath are in development, typically reporting sensitivities of 80%-88% and specificities of 70%-90%.^{131,132}

Acute phase proteins: recent studies have revisited the use of acute phase proteins such as C-reactive protein (CRP) and Alpha-1-acid glycoprotein as triage tests for TB.¹³³ Systematic reviews have described that CRP has a sensitivity of 90-95% with a specificity that ranges from 50% to 70% depending on the setting. A prospective study in HIV positive individuals recently reported 89% sensitivity results against culture, and similar sensitivity as the WHO symptom-based screening when compared to Xpert MTB/RIF.¹³³ CRP tests are already available in a portable and designed lock format and hence this is a potential marker which could be used for screening purposes within active case finding strategies.

A recent proteomic analysis of 1,470 samples from patients described six host response markers including tryptophanyl-TRNA synthetase, kallistatin, complement C9, gelsolin, testican-2, and aldolase C which had performed well in a training set to distinguish TB and non-TB samples. Differential expression was also high for many novel proteins not previously associated with TB such as SAA, secreted phospholipase A2 (NPS-PLA2), and carbonic anhydrase 6 (CA6).¹³⁴

A pleural fluid adenosine deaminase (ADA) >35 U/L has been reported to have a sensitivity of 93%, with 90% specificity for the diagnosis of TB in lymphocytic exudates. A high ADA level is also detected in neutrophilic TB effusions. However, extremely high ADA activity should raise suspicion of empyema or lymphoma.¹³⁵ As ADA levels decrease with treatment, this is a potential biomarker of disease recovery.^{136,137} ADA exceeds Xpert's sensitivity for pleural and pericardial TB,^{138,139} for which unstimulated interferon-gamma is another promising biomarker.

The combined use of ascitic fluid ADA and serum CA-125 in 30 patients reported a sensitivity, specificity of ADA of 87%.

Several other human biomarkers have decreased concentrations after TB treatment initiation¹⁴⁰ with significant correlations between CRP, serum amyloid protein A (SAA), vascular endothelial growth factor A (VEGF-A), soluble interleukin 2 receptor-alpha (sIL2R-A)/CD40, and γ -interferon (IFN) inducible protein 10 (IP10) and delayed smear culture conversion.¹⁴⁰⁻¹⁴² IP10 has been reported to be high in unstimulated plasma of children and adults with active TB.¹⁴³ A combination of monocyte chemoattractant protein-1 (MCP-1)/C-C motif chemokine 2 (CCL2), IP-10, sIL-2R α , SAA, CRP and smear microscopy smear grade could distinguish fast from slow responders and were predictive of delayed smear culture conversion with 86% and 83% sensitivity and specificity, respectively. Thus, some of these biomarkers could play a role in the identification of patients put under treatment without having TB (among those clinically diagnosed), or those with drug resistant TB put under drug susceptible TB combinations.^{144,145}

The neutrophil driven interferon (IFN)-inducible gene profile (Type 2 [IFN γ] and Type I [IFN $\alpha\beta$] IFN signalling) has a TB signature detectable in the peripheral blood and its levels decrease with effective treatment.¹⁴⁶

The combined use of ascitic fluid ADA and serum CA-125 in 30 patients reported a sensitivity, specificity of ADA of 87% and 83% respectively, whereas CA-125 had 83% sensitivity and 50% specificity.¹⁴⁷

Table 3 Host and pathogen biomarkers with potential for POC diagnosis of Tuberculosis (Modified from Goletti, 2016).

Biomarkers	Urine	Blood (unstimulated)	Blood (stimulated)	Breath
From <i>M. tuberculosis</i>	LAM, Ag85b, DNA	DNA, Ag 85b		
From the host	IP10	MicroRNA, proteomic and metabolomic profiles, IP10, transcriptional and metabolic signatures	Phenotype by fluorescence-activated cell sorting, ELISA, transcriptional signatures	Volatile organic compounds (VOCs)

Although many of these markers are promising, none of these biomarkers, except LAM and acute phase proteins, are available in POC format and there are no prototype diagnostics at this stage.

Other novel assays

Point-of-care diagnostic development should combine the most innovative technologies with effective early detection and affordable costs.

For some time, considerable attention has been devoted in the bioelectronic field. Biosensors are analytical devices that combine a biological sensing element with a physico-chemical transducer. The importance of biosensors results from their high analytical specificity and sensitivity, which allows the detection of a broad spectrum of analytes in complex sample matrices.¹⁴⁸ Importantly, a biosensor can only (in ideal situations) perform as well as the association of the biomarker it is designed to detect with the condition of interest.

Also, other properties, such as being easy-to-operate, economical, portable and real-time results provider, identify them as promising future diagnostic methods. Nucleic acid or antibody based biosensors have been built to detect different molecular targets. There are several promising assays aimed at finding whole bacteria or defining specific MTBC antigens: an amperometric immunosensor to detect MTB cells in sputum, captured on a microtip surface and detected by electric current¹⁴⁹ or an immuno-sensor development of ESAT-6, based on a voltammetry method,¹⁵⁰ are only some of them.

The combination of nanotechnology and biosensing technology has great potential in the medical diagnostics field.¹⁴⁸ A magnetoresistive biosensor to detect BCG bacteria,¹⁵¹ a lab-on-chip (LOC) platform which can perform label-free and rapid single-cell capture¹⁵² or a colorimetric sensing strategy employing gold nanoparticles¹⁵³ are very promising assays.

Other tantalizing developments with potential for POC could be transcriptional signatures of host blood. An interferon-inducible neutrophil-driven blood transcriptional signature distinguished pulmonary tuberculosis from other respiratory or granulomatous diseases or cancers.¹⁵⁴ Other relevant study showed that host mRNA signature from host blood can differentiate tuberculosis from other diseases in several cohorts of African children, regardless of HIV infection.¹⁵⁵ However, the equipment and complexity involved in the analysis of transcriptional signatures preclude their use in POC for now.

Conclusion

Although we are emerging from a period of unprecedented research, development, and implementation for new TB diagnostics, we have a limited number of near POC tests, and even fewer commercially-available true POC tests. None of these meet any of the WHO TPP criteria but they still are useful and, in the case of LF-LAM for example, have compelling evidence to support their implementation. Furthermore, there is a worrying gap of late stage potential POC tests for TB. Finally, not only can the simplicity of tests

be improved so they are deployable at the POC, but the conditions at the POC can themselves be raised, so that more sophisticated tests are doable. This requires a holistic approach to ending TB.

Conflicts of interest

GT has received consumables donations from Cepheid, Alere, and Hain LifeSciences. GT has consulted for the WHO.

Acknowledgements

The paper is part of the ERS/ALAT and the ERS/SBPT collaborative projects (ERS: European Respiratory Society; ALAT: Latino-American Society of Respiratory Medicine; SBPT: Brazilian Society of Pulmonology).

References

1. World Health Organization. Global tuberculosis report 2017. Geneva, Switzerland: WHO/HTM/TB; 2017.
2. Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias HM, et al. WHO's new End TB Strategy. *Lancet*. 2015;385:1799–801.
3. Schroeder L, Amukele T, Pai M. 2016. <https://www.forbes.com/sites/sciencebiz/2016/08/04/why-the-world-needs-an-essential-diagnostics-list/#12ccc2534015> (accessed 01.11.17).
4. Denkinger CM, Nicolau I, Ramsay A, Chedore P, Pai M. Are peripheral microscopy centres ready for next generation molecular tuberculosis diagnostics? *Eur Respir J*. 2013;42:544–7.
5. Global Tuberculosis Programme. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance.
6. World Health Organization. Xpert MTB/FIR assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Geneva; 2014.
7. World Health Organization (WHO). The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. Policy guidance; 2015.
8. World Health Organization. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. Geneva, Switzerland; 2017.
9. Schito M, Peter TF, Cavanaugh S, Piatek AS, Young GJ, Alexander H, et al. Opportunities and challenges for cost-efficient implementation of new point-of-care diagnostics for HIV and tuberculosis. *J Infect Dis*. 2012;205 Suppl. 2:S169–80.
10. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva, Switzerland: WHO/HTM/TB/2014.18; 2014.
11. Karen RS, Megan H, Vivienne N, Philip CH, Andrew R, Jane C, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6:570–81.
12. Lumb R, Van Deun A, Bastian I, Fitz-Gerald M. Sputum microscopy the handbook; 2013.
13. Alarcon E, Asokan, Broekmans J, Caminero J. Tuberculosis care TREATMENT endorsements; 2006.
14. Controlpoint D. Technical guide; 2000.
15. Singhal R, Myneedu VP. Microscopy as a diagnostic tool in pulmonary tuberculosis. *Int J Mycobacteriol*. 2015;4:1–6.
16. Whitelaw A, Peter J, Sohn H, Viljoen D, Theron G, Badri M, et al. Comparative cost and performance of light-emitting

- diode microscopy in HIV-tuberculosis-co-infected patients. *Eur Respir J*. 2011;38:1393–7.
17. Global Laboratory Initiative, Stop TB Partnership. Laboratory diagnosis of tuberculosis by sputum microscopy: the handbook; 2013. [http://www.stoptb.org/wg/gli/assets/documents/TB MICROSCOPY HANDBOOK_FINAL.pdf](http://www.stoptb.org/wg/gli/assets/documents/TB_MICROSCOPY_HANDBOOK_FINAL.pdf) (accessed 01.11.17).
 18. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48:229–37.
 19. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert[®] MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev*. 2014;1:CD009593.
 20. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *Eur Respir J*. 2014;44:435–46.
 21. García-Basteiro AL, Ismail MR, Carrilho C, Ussene E, Castillo P, Chitsungo D, et al. The role of Xpert MTB/RIF in diagnosing pulmonary tuberculosis in post-mortem tissues; 2016, <http://dx.doi.org/10.1038/srep20703>.
 22. UNITAID, World Health Organization. Tuberculosis diagnostic technology landscape; 2017. <https://unitaid.eu/assets/2017-Unitaid-TB-Diagnostics-Technology-Landscape.pdf> (accessed 25.10.17).
 23. Dheda K, Ruhwald M, Theron G, Peter J, Yam WC. Point-of-care diagnosis of tuberculosis: past, present and future. *Respirology*. 2013;18:217–32.
 24. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. *Lancet*. 2013;383:424–35.
 25. Hanrahan CF, Clouse K, Bassett J, Mutunga L, Selibas K, Stevens W, et al. The patient impact of point-of-care vs. laboratory placement of Xpert[®] MTB/RIF. *Int J Tuberc Lung Dis*. 2015;19:811–6.
 26. Hanrahan CF, Selibas K, Deery CB, Dansey H, Clouse K, Bassett J, et al. Time to treatment and patient outcomes among TB suspects screened by a single point-of-care Xpert MTB/RIF at a primary care clinic in Johannesburg, South Africa. *PLOS ONE*. 2013;8:e65421.
 27. Clouse K, Page-Shipp L, Dansey H, Moatlhodi B, Scott L, Bassett J, et al. Implementation of Xpert MTB/RIF for routine point-of-care diagnosis of tuberculosis at the primary care level. *South African Med J*. 2012;102:805.
 28. Schnippel K, Meyer-Rath G, Long L, MacLeod W, Sanne I, Stevens WS, et al. Scaling up Xpert MTB/RIF technology: the costs of laboratory- vs. clinic-based roll-out in South Africa. *Trop Med Int Health*. 2012;17:1142–51.
 29. Theron G. Point-of-care technologies for the diagnosis of active tuberculosis; 2016. p. 556–79.
 30. Lessells RJ, Cooke GS, McGrath N, Nicol MP, Newell M-L, Godfrey-Faussett P. Impact of point-of-care Xpert MTB/RIF on tuberculosis treatment initiation. A cluster-randomized trial. *Am J Respir Crit Care Med*. 2017;196:901–10.
 31. Agizew T, Boyd R, Ndwapi N, Auld A, Basotli J, Nyirenda S, et al. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic, stepped-wedge trial in Botswana. *PLOS ONE*. 2017;12:e0183237.
 32. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. *Lancet Glob Health*. 2015;3:e450–7.
 33. Cox HS, Mbhele S, Mohess N, Whitelaw A, Muller O, Zemanay W, et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. *PLoS Med*. 2014;11:e1001760.
 34. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? *Lancet Infect Dis*. 2014;14:527–32.
 35. Theron G, Peter J, Van Zyl-Smit R, Mishra H, Streicher E, Murray S, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *Am J Respir Crit Care Med*. 2011;184:132–40.
 36. Theron G, Peter J, Calligaro G, Meldau R, Hanrahan C, Khalfey H, et al. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for TB diagnosis using specimens from different body compartments. *Sci Rep*. 2014;4:1–10.
 37. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. *Lancet Respir Med*. 2015;3:451–61.
 38. Theron G. Xpert MTB/RIF to diagnose tuberculosis in children. *Lancet Respir Med*. 2015;3:419–21.
 39. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *Lancet Respir Med*. 2017;5:291–360.
 40. Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *Eur Respir J*. 2016;48:516–25.
 41. Kik SV, Denkinger CM, Chedore P, Pai M. Replacing smear microscopy for the diagnosis of tuberculosis: what is the market potential? *Eur Respir J*. 2014;43:1793–6.
 42. Raizada N, Sachdeva KS, Sreenivas A, Vadera B, Gupta RS, Parmar M, et al. Feasibility of decentralised deployment of Xpert MTB/RIF test at lower level of health system in India. *PLOS ONE*. 2014;9:e89301.
 43. Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, et al. Xpert MTB/RIF results in patients with previous tuberculosis: can we distinguish true from false positive results? *Clin Infect Dis*. 2016;62:995–1001.
 44. Theron G, Venter R, Smith L, Esmail A, Randall P, Sood V, et al. False positive Xpert MTB/RIF results in re-tested patients with previous tuberculosis: frequency, profile, and prospective clinical outcomes. *J Clin Microbiol*. 2018;JCM-01696.
 45. South African Department of Health. National tuberculosis treatment guidelines; 2014. http://www.sahivsoc.org/upload/documents/NTCP_Adult_TB_Guidelines_27.5.2014.pdf (accessed 25.10.17).
 46. Venter R, Derendinger B, de Vos M, Pillay S, Dolby T, Simpson J, et al. Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping. *Sci Rep*. 2017;7:14854.
 47. World Health Organization. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF; 2017. p. 1–11.
 48. García-Basteiro AL, Saavedra B, Cobelens F. The good, the bad and the ugly of the next-generation Xpert Mtb/Rif[®] Ultra test for tuberculosis diagnosis. *Arch Bronconeumol*. 2017, <http://dx.doi.org/10.1016/j.arbres.2017.05.023>.
 49. Hamasur B, Bruchfeld J, Haile M, Pawlowski A, Bjorvatn B, Källenius G, et al. Rapid diagnosis of tuberculosis by

- detection of mycobacterial lipoarabinomannan in urine. *J Microbiol Methods*. 2001;45:41–52.
50. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. *Lancet Infect Dis*. 2012;12:201–9.
 51. Peter JG, Theron G, van Zyl-Smit R, Haripersad A, Mottay L, Kraus S, et al. Diagnostic accuracy of a urine lipoarabinomannan strip-test for TB detection in HIV-infected hospitalised patients. *Eur Respir J*. 2012;40:1211–20.
 52. Kroidl I, Clowes P, Reither K, Mtafya B, Rojas-Ponce G, Ntinginya EN, et al. Performance of urine lipoarabinomannan assays for paediatric tuberculosis in Tanzania. *Eur Respir J*. 2015;46:761–70.
 53. Lacourse SM, Pavlinac PB, Cranmer LM, Njuguna IN, Mugo C, Gatimu J, et al. Stool Xpert MTB/RIF and urine lipoarabinomannan (LAM) for diagnosing tuberculosis in hospitalized HIV-infected children. *AIDS*. 2017:1.
 54. Lawn SD, Dheda K, Kerkhoff AD, Peter JG, Dorman S, Boehme CC, et al. Determine TB-LAM lateral flow urine antigen assay for HIV-associated tuberculosis: recommendations on the design and reporting of clinical studies. *BMC Infect Dis*. 2013;13:407.
 55. Kerkhoff AD, Lawn SD. A breakthrough urine-based diagnostic test for HIV-associated tuberculosis. *Lancet*. 2016;387:1139–41.
 56. Lawn SD, Kerkhoff AD, Burton R, Schutz C, Boule A, Vogt M, 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;15:67.
 57. Peter J, Theron G, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Test characteristics and potential impact of the urine LAM lateral flow assay in HIV-infected outpatients under investigation for TB and able to self-expectorate sputum for diagnostic testing. *BMC Infect Dis*. 2015;15:262.
 58. Bjerrum S, Kenu E, Lartey M, Newman MJ, Addo KK, Andersen AB, et al. Diagnostic accuracy of the rapid urine lipoarabinomannan test for pulmonary tuberculosis among HIV-infected adults in Ghana – findings from the DETECT HIV-TB study. *BMC Infect Dis*. 2015;15:407.
 59. Huerga H, Ferlazzo G, Belvacqua P, Kirubi B, Ardizzoni E, Wanjala S, 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;12:1–15.
 60. Gupta-Wright A, Peters JA, Flach C, Lawn SD. Detection of lipoarabinomannan (LAM) in urine is an independent predictor of mortality risk in patients receiving treatment for HIV-associated tuberculosis in sub-Saharan Africa: a systematic review and meta-analysis. *BMC Med*. 2016;14:53.
 61. Lawn SD. Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. *BMC Infect Dis*. 2012;12:103.
 62. Balcha TT, Winqvist N, Sturegård E, Skogmar S, Reepalu A, Jemal ZH, et al. Detection of lipoarabinomannan in urine for identification of active tuberculosis among HIV-positive adults in Ethiopian health centres. *Trop Med Int Heal*. 2014;19:734–42.
 63. Drain PK, Gounder L, Grobler A, Sahid F, Bassett IV, Moosa M-YS. Urine lipoarabinomannan to monitor antituberculosis therapy response and predict mortality in an HIV-endemic region: a prospective cohort study. *BMJ Open*. 2015;5:e006833.
 64. Suwanpimolkul G, Kawkitinarong K, Manosuthi W, Sophonphan J, Gatechompol S, Ohata PJ, 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;59:96–102.
 65. Holtz TH, Kabera G, Mthiyane T, Zingoni T, Nadesan S, Ross D, et al. Use of a WHO-recommended algorithm to reduce mortality in seriously ill patients with HIV infection and smear-negative pulmonary tuberculosis in South Africa: an observational cohort study. *Lancet Infect Dis*. 2011;11:533–40.
 66. Lawn SD, Edwards DJ, Kranzer K, Vogt M, Bekker L-G, Wood R. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. *AIDS*. 2009;23:1875–80.
 67. Cohen T, Murray M, Wallengren K, Alvarez GG, Samuel EY, Wilson D. The prevalence and drug sensitivity of tuberculosis among patients dying in hospital in kwazulu-natal South Africa: a postmortem study. *PLoS Med*. 2010;7, <http://dx.doi.org/10.1371/journal.pmed.1000296>.
 68. Drain PK, Gounder L, Sahid F, Moosa M-YS, Rapid Urine LAM. Testing improves diagnosis of expectorated smear-negative pulmonary tuberculosis in an HIV-endemic region. *Sci Rep*. 2016;6:19992.
 69. Peter JG, Theron G, Muchinga TE, Govender U, Dheda K. The diagnostic accuracy of urine-based Xpert MTB/RIF in HIV-infected hospitalized patients who are smear-negative or aputum scarce. *PLoS ONE*. 2012;7, <http://dx.doi.org/10.1371/journal.pone.0039966>.
 70. Peter JG, Zijenah LS, Chanda D, Clowes P, Lesosky M, Gina P, et al. Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial. *Lancet*. 2016;387:1187–97.
 71. Cox JA, Lukande RL, Kalungi S, Van Marck E, Van De Vijver K, Kambugu A, et al. Is urinary lipoarabinomannan the result of renal tuberculosis? Assessment of the renal histology in an autopsy cohort of ugandan HIV-infected adults. *PLOS ONE*. 2015;10:1–13.
 72. Lawn SD, Gupta-Wright A. Detection of lipoarabinomannan (LAM) in urine is indicative of disseminated TB with renal involvement in patients living with HIV and advanced immunodeficiency: evidence and implications. *Trans R Soc Trop Med Hyg*. 2016;3:180–5.
 73. Nakiyingi L, Moodley VM, Manabe YC, Nicol MP, Holshouser M, Armstrong DT, et al. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. *J Acquir Immune Defic Syndr*. 2014;66:270–9.
 74. Peter JG, Theron G, Dheda K. Can point-of-care urine LAM strip testing for tuberculosis add value to clinical decision making in hospitalised HIV-infected persons? *PLOS ONE*. 2013;8, <http://dx.doi.org/10.1371/journal.pone.0054875>.
 75. Hanifa Y, Fielding KL, Chihota VN, Adonis L, Charalambous S, Karstaedt A, et al. Diagnostic accuracy of lateral flow urine LAM assay for TB screening of adults with advanced immunosuppression attending routine HIV care in South Africa. *PLOS ONE*. 2016;11:1–12.
 76. Floridia M, Ciccacci F, Andreotti M, Hassane A, Sidumo Z, Magid NA, et al. Tuberculosis case finding with combined rapid point-of-care assays (Xpert MTB/RIF and determine TB LAM) in HIV-positive individuals starting antiretroviral therapy in Mozambique. *Clin Infect Dis*. 2017, <http://dx.doi.org/10.1093/cid/cix641>.
 77. Drain PK, Losina E, Coleman SM, Giddy J, Ross D, Katz JN, et al. Diagnostic accuracy of a point-of-care urine test for tuberculosis screening among newly-diagnosed HIV-infected adults: a prospective, clinic-based study. *BMC Infect Dis*. 2014;14:110.
 78. Gupta-Wright A, Fielding KL, van Oosterhout JJ, Wilson DK, Corbett EL, Flach C, et al. Rapid urine-based screening for tuberculosis to reduce AIDS-related mortality in hospitalized

- patients in Africa (the STAMP trial): study protocol for a randomised controlled trial. *BMC Infect Dis.* 2016;16:501.
79. van't Hoog AH, Meme HK, Laserson KF, Agaya JA, Muchiri BG, Githui WA, et al. Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. *PLoS ONE.* 2012;7:1–9.
 80. Theron G, Pooran A, Peter J, van Zyl-Smit R, Kumar Mishra H, Meldau R, et al. Do adjunct tuberculosis tests, when combined with Xpert MTB/RIF, improve accuracy and the cost of diagnosis in a resource-poor setting? *Eur Respir J.* 2012;40:161–8.
 81. Philipsen RHHM, Sánchez CI, Maduskar P, Melendez J, Peters-Bax L, Peter JG, et al. Automated chest-radiography as a triage for Xpert testing in resource-constrained settings: a prospective study of diagnostic accuracy and costs. *Sci Rep.* 2015;5:12215.
 82. Hogeweg L, Sánchez CI, Maduskar P, Philipsen R, Story A, Dawson R, et al. Automatic detection of tuberculosis in chest radiographs using a combination of textural focal, and shape abnormality analysis. *IEEE Trans Med Imaging.* 2015;34:2429–42.
 83. Story A, Aldridge RW, Abubakar I, Stagg HR, Lipman M, Watson JM, et al. Active case finding for pulmonary tuberculosis using mobile digital chest radiography: an observational study. *Int J Tuberc Lung Dis.* 2012;16:1461–7.
 84. Jensen SG, Olsen NW, Seersholm N, Lillebaek T, Wilcke T, Pedersen MK, et al. Screening for TB by sputum culture in high-risk groups in Copenhagen Denmark: a novel and promising approach. *Thorax.* 2015;70:979–83.
 85. Heuvelings CC, de Vries SG, Greve PF, Visser BJ, Bélard S, Janssen S, et al. Effectiveness of interventions for diagnosis and treatment of tuberculosis in hard-to-reach populations in countries of low and medium tuberculosis incidence: a systematic review. *Lancet Infect Dis.* 2017;17:e144–58.
 86. Datta B, Hazarika A, Shewade HD, Ayyagari K, Kumar AMV. Digital chest X-ray through a mobile van: public private partnership to detect sputum negative pulmonary TB. *BMC Res Notes.* 2017;10:96.
 87. Binopal G, Agarwal P, Kaur N, Singh B, Bhagat V, Verma RP, et al. Screening difficult-to-reach populations for tuberculosis using a mobile medical unit, Punjab, India. *Public Heal Action.* 2015;5:241–5.
 88. Myint O, Saw S, Isaakidis P, Khogali M, Reid A, Hoa NB, et al. Active case-finding for tuberculosis by mobile teams in Myanmar: yield and treatment outcomes. *Infect Dis Poverty.* 2017;6:77.
 89. Morishita F, Garfin AMCG, Lew W, Oh KH, Yadav RP, Reston JC, et al. Bringing state-of-the-art diagnostics to vulnerable populations: the use of a mobile screening unit in active case finding for tuberculosis in Palawan, the Philippines. *PLOS ONE.* 2017;12:e0171310.
 90. Ongen G, Borekci S, Icmeli OS, Birgen N, Karagul G, Akgun S, et al. Pulmonary tuberculosis incidence in Turkish prisons: importance of screening and case finding strategies. *Tuberc Toraks.* 2013;61:21–7.
 91. Abboud S, Weiss F, Siegel E, Jeudy J. TB or not TB: interreader and intrareader variability in screening diagnosis on an iPad versus a traditional display. *J Am Coll Radiol.* 2013;10:42–4.
 92. Melendez J, Sánchez CI, Philipsen RHHM, Maduskar P, Dawson R, Theron G, et al. An automated tuberculosis screening strategy combining X-ray-based computer-aided detection and clinical information. *Sci Rep.* 2016;6:25265.
 93. Maduskar P, Muyoyeta M, Ayles H, Hogeweg L, Peters-Bax L, van Ginneken B. Detection of tuberculosis using digital chest radiography: automated reading vs. interpretation by clinical officers. *Int J Tuberc Lung Dis.* 2013;17:1613–20.
 94. Muyoyeta M, Maduskar P, Moyo M, Kasese N, Milimo D, Spooner R, et al. The sensitivity and specificity of using a computer aided diagnosis program for automatically scoring chest X-rays of presumptive TB patients compared with Xpert MTB/RIF in Lusaka Zambia. *PLOS ONE.* 2014;9:16–8.
 95. Van Rie A. Xpert MTB/RIF: a game changer for the diagnosis of pulmonary tuberculosis in children? *Lancet Glob Heal.* 2013;1:e60–1.
 96. Iruedo J, O'Mahony D, Mabunda S, Wright G, Cawe B. The effect of the Xpert MTB/RIF test on the time to MDR-TB treatment initiation in a rural setting: a cohort study in South Africa's Eastern Cape Province. *BMC Infect Dis.* 2017;17:91.
 97. Hinić V, Feuz K, Turan S, Berini A, Frei R, Pfeifer K, et al. Clinical evaluation of the Abbott RealTime MTB Assay for direct detection of Mycobacterium tuberculosis-complex from respiratory and non-respiratory samples. *Tuberculosis (Edinb).* 2017;104:65–9.
 98. Yan L, Xiao H, Zhang Q. Systematic review: comparison of Xpert MTB/RIF LAMP and SAT methods for the diagnosis of pulmonary tuberculosis. *Tuberculosis (Edinb).* 2016;96:75–86.
 99. Nagai K, Horita N, Yamamoto M, Tsukahara T, Nagakura H, Tashiro K, et al. Diagnostic test accuracy of loop-mediated isothermal amplification assay for Mycobacterium tuberculosis: systematic review and meta-analysis. *Sci Rep.* 2016;6:39090.
 100. Aryan E, Makvandi M, Farajzadeh A, Huygen K, Alvandi A-H, Gouya M-M, et al. Clinical value of IS6110-based loop-mediated isothermal amplification for detection of Mycobacterium tuberculosis complex in respiratory specimens. *J Infect.* 2013;66:487–93.
 101. Kamphée H, Chaiprasert A, Prammananan T, Wiriyaichaiporn N, Kanchanatavee A, Dharakul T. Rapid molecular detection of multidrug-resistant tuberculosis by PCR-nucleic acid lateral flow immunoassay. *PLOS ONE.* 2015;10:e0137791.
 102. Bholla M, Kapalata N, Masika E, Chande H, Jugheli L, Sasamalo M, et al. Evaluation of Xpert® MTB/RIF and Ustar EasyNAT™ TB IAD for diagnosis of tuberculous lymphadenitis of children in Tanzania: a prospective descriptive study. *BMC Infect Dis.* 2016;16:246.
 103. Shenai S, Armstrong DT, Valli E, Dolinger DL, Nakiyingi L, Dietze R, et al. Analytical and clinical evaluation of the episteme genedrive assay for detection of Mycobacterium tuberculosis. *J Clin Microbiol.* 2016;54:1051–7.
 104. DiNardo AR, Hahn A, Leyden J, Stager C, Jo Baron E, Graviss EA, et al. Use of string test and stool specimens to diagnose pulmonary tuberculosis. *Int J Infect Dis.* 2015;41:50–2.
 105. Marcy O, Ung V, Goyet S, Borand L, Msellati P, Tejiokem M, et al. Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children. *Clin Infect Dis.* 2016;62:1161–8.
 106. Nicol MP, Spiers K, Workman L, Isaacs W, Munro J, Black F, et al. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. *Clin Infect Dis.* 2013;57:e18–21.
 107. Taylor N, Gaur RL, Baron EJ, Banaei N. Can a simple flotation method lower the limit of detection of Mycobacterium tuberculosis in extrapulmonary samples analyzed by the GeneXpert MTB/RIF assay? *J Clin Microbiol.* 2012;50:2272–6.
 108. Walters E, Gie RP, Hesselting AC, Friedrich SO, Diacon AH, Gie RP. Rapid diagnosis of pediatric intrathoracic tuberculosis from stool samples using the Xpert MTB/RIF assay. *Pediatr Infect Dis J.* 2012;31:1316.
 109. Xie YL, Chakravorty S, Armstrong DT, Hall SL, Via LE, Song T, et al. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. *N Engl J Med.* 2017;377:1043–54.
 110. Wallis RS, Maeurer M, Mwaba P, Chakaya J, Rustomjee R, Migliori GB, et al. Tuberculosis—advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. *Lancet Infect Dis.* 2016;16:e34–46.
 111. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol MET-AL>. Rapid molecular TB diagnosis: evidence,

- policy making and global implementation of Xpert MTB/RIF. *Eur Respir J.* 2013;42:252–71.
112. Caminero JA, Scardigli A. Classification of antituberculosis drugs: a new proposal based on the most recent evidence. *Eur Respir J.* 2015;46:887–93.
 113. Sotgiu G, Tiberi S, D'Ambrosio L, Centis R, Alffenaar JW, Caminero JA, et al. Faster for less: the new 'shorter' regimen for multidrug-resistant tuberculosis. *Eur Respir J.* 2016;48:1503–7.
 114. Caminero JA, Piubello A, Scardigli A, Migliori GB. Proposal for a standardised treatment regimen to manage pre- and extensively drug-resistant tuberculosis cases. *Eur Respir J.* 2017;50:1700648.
 115. Miotto P, Tessema B, Tagliani E, Chindelevitch L, Starks A, Emerson C, et al. A standardised method for interpreting the association between mutations and phenotypic drug-resistance in *Mycobacterium tuberculosis*. *Eur Respir J.* 2018;50.
 116. Dowdy DW, Theron G, Tornheim JA, Warren R, Kendall EA. Of testing and treatment: implications of implementing new regimens for multidrug-resistant tuberculosis. *Clin Infect Dis.* 2017;65:1206–11.
 117. Tomasicchio M, Theron G, Pietersen E, Streicher E, Stanley-Josephs D, van Helden P, et al. The diagnostic accuracy of the MTBDRplus and MTBDRsl assays for drug-resistant TB detection when performed on sputum and culture isolates. *Sci Rep.* 2016;6:17850.
 118. Pankhurst LJ, Del Ojo Elias C, Votintseva AA, Walker TM, Cole K, Davies J, et al. Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. *Lancet Respir Med.* 2016;4:49–58.
 119. Brown AC, Bryant JM, Einer-Jensen K, Holdstock J, Houniet DT, Chan JZM, et al. Rapid whole-genome sequencing of mycobacterium tuberculosis isolates directly from clinical samples. *J Clin Microbiol.* 2015;53:2230–7.
 120. Votintseva AA, Bradley P, Pankhurst L, Del Ojo Elias C, Loose M, Nilgiriwala K, et al. Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. *J Clin Microbiol.* 2017;55:1285–98.
 121. Colman RE, Anderson J, Lemmer D, Lehmkuhl E, Georghiou SB, Heaton H, et al. Rapid drug susceptibility testing of drug-resistant mycobacterium tuberculosis isolates directly from clinical samples by use of amplicon sequencing: a proof-of-concept study. *J Clin Microbiol.* 2016;54:2058–67.
 122. Jeanes C, O'Grady J. Diagnosing tuberculosis in the 21st century – dawn of a genomics revolution? *Int J Mycobacteriol.* 2016;5:384–91.
 123. Wlodarska M, Johnston JC, Gardy JL, Tang P. A microbiological revolution meets an ancient disease: improving the management of tuberculosis with genomics. *Clin Microbiol Rev.* 2015;28:523–39.
 124. McNerney R, Clark TG, Campino S, Rodrigues C, Dolinger D, Smith L, 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;56:130–5.
 125. World Health Organization, International Programme on Chemical Safety. Biomarkers in risk assessment: validity and validation; 2001. <http://www.inchem.org/documents/ehc/ehc/ehc222.htm> (accessed 01.10.17).
 126. Goletti D, Petruccioli E, Joosten SA, Ottenhoff THM. Tuberculosis biomarkers: from diagnosis to protection. *Infect Dis Rep.* 2016;8, <http://dx.doi.org/10.4081/idr.2016.6568>.
 127. du Preez I, Lues L, Loots DT. Metabolomics biomarkers for tuberculosis diagnostics: current status and future objectives. *Biomark Med.* 2017;11:179–94.
 128. Yerlikaya S, Broger T, MacLean E, Pai M, Denkinger CM. A tuberculosis biomarker database: the key to novel TB diagnostics. *Int J Infect Dis.* 2017;56:253–7.
 129. Kashyap RS, Saha SM, Nagdev KJ, Kelkar SS, Purohit HJ, Taori GM, et al. Diagnostic markers for tuberculosis ascites: a preliminary study. *Biomark Insights.* 2010;5:87–94.
 130. Kashyap RS, Rajan AN, Ramteke SS, Agrawal VS, Kelkar SS, Purohit HJ, et al. Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study. *BMC Infect Dis.* 2007;7:74.
 131. Coronel Teixeira R, Rodríguez M, Jiménez de Romero N, Bruins M, Gómez R, Yntema JB, et al. The potential of a portable, point-of-care electronic nose to diagnose tuberculosis. *J Infect.* 2017, <http://dx.doi.org/10.1016/j.jinf.2017.08.003>.
 132. Bruins M, Rahim Z, Bos A, van de Sande WWJ, Endtz HP, van Belkum A. Diagnosis of active tuberculosis by e-nose analysis of exhaled air. *Tuberculosis.* 2013;93:232–8.
 133. Yoon C, Semitala FC, Atuhumuza E, Katende J, Mwebe S, Asege L<ET-LA>. Point-of-care C-reactive protein-based tuberculosis screening for people living with HIV: a diagnostic accuracy study. *Lancet Infect Dis.* 2017, [http://dx.doi.org/10.1016/S1473-3099\(17\)30488-7](http://dx.doi.org/10.1016/S1473-3099(17)30488-7).
 134. De Groot MA, Sterling DG, Hraha T, Russell TM, Green LS, Wall K, et al. Discovery and validation of a six-marker serum protein signature for the diagnosis of active pulmonary tuberculosis. *J Clin Microbiol.* 2017;55:3057–71.
 135. Porcel JM, Esquerda A, Bielsa S. Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2100 consecutive patients. *Eur J Intern Med.* 2010;21:419–23.
 136. Ige O, Edem VF, Arinola OG. Plasma adenosine deaminase enzyme reduces with treatment of pulmonary tuberculosis in Nigerian patients: indication for diagnosis and treatment monitoring. *Niger J Physiol Sci.* 2016;31:49–53.
 137. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, et al. Serum adenosine deaminase: a novel biomarker tool for the diagnosis of tuberculosis. *Biosci Biotechnol Res Asia.* 2016;13:551–6.
 138. Pandie S, Peter JG, Kerbelker ZS, Meldau R, Theron G, Govender U, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon- γ in a high burden setting: a prospective study. *BMC Med.* 2014;12:101.
 139. Meldau R, Peter J, Theron G, Calligaro G, Allwood B, Symons G, et al. Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN- γ for the evaluation of pleural tuberculosis: a prospective cohort study. *BMC Pulm Med.* 2014;14:58.
 140. Ferrian S, Manca C, Lubbe S, Conradie F, Ismail N, Kaplan G, et al. A combination of baseline plasma immune markers can predict therapeutic response in multidrug resistant tuberculosis. *PLOS ONE.* 2017;12:e0176660.
 141. Riou C, Perez Peixoto B, Roberts L, Ronacher K, Walzl G, Manca C, et al. Effect of standard tuberculosis treatment on plasma cytokine levels in patients with active pulmonary tuberculosis. *PLoS ONE.* 2012;7:e36886.
 142. Jayakumar A, Vittinghoff E, Segal MR, MacKenzie WR, Johnson JL, Gitta P, et al. Serum biomarkers of treatment response within a randomized clinical trial for pulmonary tuberculosis. *Tuberculosis.* 2015;95:415–20.
 143. Wergeland I, Pullar N, Assmus J, Ueland T, Tonby K, Feruglio S, et al. IP-10 differentiates between active and latent tuberculosis irrespective of HIV status and declines during therapy. *J Infect.* 2015;70:381–91.
 144. García-Basteiro AL, Mambuque E, den Hertog A, Saavedra B, Cuamba I, Oliveras L, et al. IP-10 kinetics in the first week of therapy are strongly associated with bacteriological

- confirmation of tuberculosis diagnosis in HIV-infected patients. *Sci Rep*. 2017;7:14302.
145. den Hertog AL, Mayboroda OA, Klatser PR, Anthony RM. Simple rapid near-patient diagnostics for tuberculosis remain elusive – is a ‘treat-to-test’ strategy more realistic? *PLoS Pathog*. 2011;7:e1002207.
 146. Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*. 2010;466:973–7.
 147. Ali N, Nath NC, Parvin R, Rahman A, Bhuiyan TM, Rahman M, et al. Role of ascitic fluid adenosine deaminase (ADA) and serum CA-125 in the diagnosis of tuberculous peritonitis. *Bangladesh Med Res Counc Bull*. 2014;40:89–91.
 148. He X, Zhou L, He D, Wang K, Qin D. Biosensing technologies for mycobacterium tuberculosis detection: status and new developments. *Clin Dev Immunol*. 2011;2011, <http://dx.doi.org/10.1155/2011/193963>.
 149. Hiraiwa M, Kim J, Lee H, Inoue S, Becker AL, Weigel KM, et al. *Mycobacterium tuberculosis*; 2016. p. 1–20.
 150. Diouani MF, Ouerghi O, Refai A, Belgacem K, Tlili C, Laouini D, et al. Detection of ESAT-6 by a label free miniature immunoelectrochemical biosensor as a diagnostic tool for tuberculosis. *Mater Sci Eng C*. 2017;74:465–70.
 151. Barroso TG, Martins RC, Fernandes E, Cardoso S, Rivas J, Freitas PP. Detection of BCG bacteria using a magnetoresistive biosensor: a step towards a fully electronic platform for tuberculosis point-of-care detection. *Biosens Bioelectron*. 2017, <http://dx.doi.org/10.1016/j.bios.2017.09.004>.
 152. Esfandyarpour R, DiDonato MJ, Yang Y, Durmus NG, Harris JS, Davis RW. Multifunctional, inexpensive, and reusable nanoparticle-printed biochip for cell manipulation and diagnosis. *Proc Natl Acad Sci USA*. 2017;114:E1306–15.
 153. Tsai T-T, Huang C-Y, Chen C-A, Shen S-W, Wang M-C, Cheng C-M, et al. Diagnosis of tuberculosis using colorimetric gold nanoparticles on a paper-based analytical device. *ACS Sens*. 2017, <http://dx.doi.org/10.1021/acssensors.7b00450>.
 154. Bloom CI, Graham CM, Berry MPR, Rozakeas F, Redford PS, Wang Y, et al. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. *PLOS ONE*. 2013;8:e70630.
 155. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of childhood tuberculosis and host RNA expression in Africa. *N Engl J Med*. 2014;370:1712–23.
 156. World Health Organization. Implementing the End TB strategy: the essentials. Geneva, Switzerland; 2015. http://www.who.int/tb/publications/2015/The_Essentials_to_End_TB/en/