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The Tuberculosis Diagnostics Pipeline

By Mark Harrington

> *That things just go on like this is the catastrophe.*
>
> —Walter Benjamin[1]

Introduction

Because of the lack of effective, accessible point-of-care (POC) tests for all forms of tuberculosis (TB), 1.5 million people die of this treatable, usually curable disease each year. Annually, 3 million, or one-third of all, TB cases are never detected, reported, or properly treated. Among people with multidrug-resistant TB (MDR-TB), fewer than 20% receive proper treatment.[2] The lack of effective TB diagnosis and drug-susceptibility testing (DST) is responsible both for onward transmission of TB and for unnecessary suffering and death.

The world's failure to invest in a successful effort to render all cases of TB easily diagnosable remains baffling and infuriating. Countries and global donors are investing billions in often poorly functioning TB programs whose greatest needs – for better diagnostics, drugs, and vaccines – are being drastically underfunded by research institutions in both developed and developing countries. Treatment Action Group's most recent report on TB research and development (R&D) funding trends shows that in 2013 the world invested just US$67.77 million in TB diagnostics R&D. This represents a mere 19.9% of the annual US$340 million investment recommended by the World Health Organization (WHO) in its *Global Plan to Stop TB: 2011–2015*.[3,4] Even the few improved new technologies that have been endorsed by the WHO over the past seven years are underused and inaccessible to most people with TB today.

Last year's *Pipeline Report* described TB diagnostics research as being "at a standstill." It would be an exaggeration to say the last 12 months have seen an increase in momentum or investment. This chapter describes the noteworthy advances that have been documented in the published literature or occurred in clinical trials or policy.

Background

For the past 133 years, sputum-smear microscopy for acid-fast bacilli – of which TB is one – has been the most widely used test for TB. The test is nonspecific to TB and misses up to half of pulmonary cases – even more among children and HIV-positive people – and by definition all extrapulmonary ones. TB culture on solid media has also been used to diagnose TB for over a century and in DST since the introduction of TB chemotherapy in the 1940s. But culture on solid media can take months, meaning that results cannot be used to guide therapy at the outset. In 1993, the WHO recommended the microscopy-based DOTS strategy for worldwide TB control. One unanticipated consequence of the recommendation may have been to lead some countries to further degrade – if they had not already dismantled – their TB microbiology (culture) laboratories. In these cases, the ability to diagnose drug-resistant TB or to determine appropriate treatment was being dismantled just as the worldwide MDR-TB epidemic made its explosive debut.

In late 2006, researchers from South Africa and the United States reported an outbreak of extensively drug-resistant TB (XDR-TB) at an HIV clinic in rural KwaZulu-Natal, South Africa.[5] Activists and policy makers realized that countries needed to move fast to improve TB laboratory capacity and to modernize the diagnostics armamentarium used in medium- and low-income-country TB programs. Over the course of 2008,

groups such as the AIDS Rights Association of Southern Africa, Médecins Sans Frontières, Partners In Health, and Treatment Action Group held two workshops to highlight the need for a TB POC test and to develop target product profiles.[58] The following three years saw a surge of new WHO recommendations including:

- liquid culture media such as the mycobacterial growth indicator tube automated platform,[6]
- rapid species identification such as with the Capilia rapid speciation test to distinguish TB from nontuberculous mycobacteria (NTM),[6] and
- line probe assays for rapid detection of MDR-TB such as the GenoType MTBDR*plus* assay.[7]

These tests provided advantages over smear microscopy and solid culture. TB in liquid culture was measurable in weeks rather than months. The speciation test revealed in 20 minutes whether a culture was *Mycobacterium tuberculosis* (MTB) or NTM. The GenoType MDRTB*plus* could diagnose many forms of TB with common genetic mutations to rifampin and isoniazid – resistance to both of which was the signature of MDR-TB – within a day or two.

The WHO continued to broaden the recommended laboratory options for low- and middle-income countries with policy statements on:

- noncommercial culture and DST methods,[8]
- same-day diagnosis by microscopy,[9]
- fluorescence microscopy,[10] and
- the GeneXpert MTB/RIF (rifampin) automated, real-time, cartridge-based PCR nucleic acid amplification test (NAAT) (2010,[11] updated 2013).[12]

Increasingly, NAA-based diagnostic tests are replacing culture-based ones for many diseases and, in the form of HIV and hepatitis C virus viral-load assays, have long been the basis for clinical staging and monitoring of treatment. In only two hours, the Xpert MTB/RIF test can determine from sputum whether TB and rifampin resistance are present; Xpert has also demonstrated sensitivity and specificity using samples from nonpulmonary tissues and fluids where TB is growing (gastric juices, lymph nodes, and cerebrospinal fluid).[12a,12b]

All, however, are expensive laboratory tests requiring electricity, controlled temperature, and trained personnel, all of which are in short or erratic supply at the points of care where most people at risk for or living with TB receive their care.

The WHO also tried to simplify the lives of laboratory workers and defray unnecessary costs to patients and payers by recommending against the use of common serologic (blood) tests for TB[13] and interferon-gamma release assays (IGRAs) in low- and middle-income countries.[14]

The WHO has yet to recommend a new TB diagnostic test since Xpert (2010/2013). In 2013, expert review panels found significant flaws with both the Eiken TB-LAMP[15] (loop-mediated isothermal amplification) and the Hain Lifescience MTBDR*sl* (which aims to detect resistance to second-line fluoroquinolones and injectables) tests,[16] declined to recommend them based on insufficient evidence, and suggested additional research.

The WHO has not reviewed the MTBDR*sl* test subsequently, and results of a June 2015 review of LAMP are not yet publicly known.

In June 2015, a WHO expert group reviewed data on the Alere Determine urine lipoarabinomannan (LAM) lateral flow test. The results of this review are not yet public.

Table 1 lists TB diagnostic test candidates relatively late in development with data published since the *2014 Pipeline Report*. For an encyclopedic review of the current TB diagnostic pipeline, see the 2014 UNITAID *Tuberculosis Diagnostics Laboratory and Market Landscape, 3rd edition*.[17] More succinct overviews are available from Pai,[18] Pai and Schito,[19] and Dorman.[20] Table 2 lists other tests discussed in the *2014 Pipeline Report* with no new publications since last year's report.

Table 1. 2015 Tuberculosis Diagnostics Pipeline: Products in Later-Stage Development or on Track for Evaluation by the WHO with New Published Data Since the *2014 Pipeline Report*

Test	Type	Sponsor	Status	Comments
MOLECULAR/NAAT/DST				
BD MAX MTB assay	qPCR for MTB in automated BD MAX	Becton, Dickinson	100% sensitive/specific for smear-positive samples[27]	
EasyNAT	Isothermal DNA amplification/lateral flow to detect MTB	Ustar	Poor sensitivity, especially for smear-negative specimens, in Tanzanian field study[28]	
FluoroType MTB	Semi-automated direct MTB detection; PCR in a closed system; results in 3 hours	Hain Lifescience	Two new studies since 2014[29,30]	Marketed
GeneChip	RT-PCR for RIF + INH DR	CapitalBio	Chinese Center for Disease Control and Prevention and University of Georgia published a paper on 1,400 samples from SW China[31]	Marketed
GenoType MTBDR*sl*	Line probe assay for FQ + SLID resistance	Hain Lifescience	WHO urged further study;[16] 2014 *Cochrane* review equivocal[33]	Sponsor claims 2.0 version superior[32]
LiPA pyrazinamide	Line probe assay for PZA resistance	Nipro	Thai field study 2015[34]	Marketed. No independent studies
MeltPro TB/INH	Closed-tube RT-PCR for INH DR	Zeesan Biotech	3-site evaluation of 1,096 clinical isolates[35]	Chinese FDA-approved
MeltPro TB/STR	Closed-tube RT-PCR for streptomycin DR	Zeesan Biotech	3-site evaluation of 1,056 clinical isolates[36]	
PURE-LAMP	Manual NAAT by loop-mediated isothermal amplification for MTB detection	Eiken	June 2014;[50] WHO review June 2015	WHO review results not publicly known
RealTime MTB/TB MDx m2000	Automated RT-PCR for MTB; can be added to HIV RNA platform	Abbott	Lower limit of detection than Roche Cobas assay[38]	CE marked[37]
REBA MTB-XDR	Line-probe assay for FQ + SLID DR	YD Diagnostics	Initial study 2015[39]	Marketed
Xpert MTB/RIF Ultra	Next-generation cartridge-based detection of MTB + RIF resistance	Cepheid	Initial study CROI 2015[40]	"Data showed the new Xpert MTB/RIF Ultra test with a new sampling processing cartridge is as sensitive as liquid culture. #CROI2015 #TB"[41]
VOLATILE ORGANIC COMPOUNDS				
Giant African pouched rats (*Cricetomys gambianus*)	Trained sniffer rates to detect MTB in sputum	Apopo Foundation	Initial study 2009[42]	Rats detected 80% of MTB species while ignoring Mycobacterium avium/intracellulare[43]
AUTOMATED IMAGING				
CAD 4TB	Digital CXR for TB screening	Delft Imaging Systems	Used in ZAMSTAR study	Three new studies in 2014–2015[44,45,46]
ANTIBODY/ANTIGEN DETECTION				
Determine TB LAM Ag	Urine dipstick for TB LAM protein	Alere	Expert review for WHO, June 2015	Results of WHO review not publicly known

CE: Conformitè Européenne (a safety certification for sale in European Economic Area countries)
CROI: Conference on Retroviruses and Opportunistic Diseases
CXR: chest X-ray
DR: drug resistance
EMB: ethambutol
FQ: fluoroquinolone
INH: isoniazid
MTB: *Mycobacterium tuberculosis*
NAAT: nucleic acid amplification test
PZA: pyrazinamide
RIF: rifampin
RT-PCR: real-time polymerase chain reaction
SLID: second-line injectable drug (e.g., amikacin, capreomycin, or kanamycin)
STR: streptomycin

Table 2. Later-Stage or Marketed TB Diagnostic Test Candidates with No New Published Data

Test	Type	Sponsor	Last Published Paper(s)	Comments
MOLECULAR/NAAT				
FluoroType MTB RNA	MTB RNA for monitoring of anti-TB therapy	Hain Lifescience	N/A	No published data
Genedrive MTB/RIF	Portable RT-PCR for MTB + RIF resistance	Epistem/Foundation for Innovative New Diagnostics, Boston University, the Johns Hopkins University	2014[47]	Licensed in E.U., India; comparative NCT02252198 study under way
LATE-PCR with Lights-On/ Lights-Off Probes + PrimeSafe	Single-tube PCR to detect MTB, resistance to INH, RIF, EMB, SLID	Hain Lifescience/Brandeis University, Stellenbosch University	2012[48]	No published data on TB application
LiPA MDR-TB	Line probe assay for RIF + INH resistance	Nipro	2013[49]	Marketed. No independent studies
REBA MTB-MDR	Line probe assay for RIF + INH resistance	YD Diagnostics	2013[51]	Marketed. One published study[51]
TRC Rapid MTB	Automated rapid rRNA to detect MTB	Tosoh	2010[52]	"Tosoh's molecular testing systems for tuberculosis…are exponentially faster than traditional methods"[53]
Truenat MTB	Chip-based NAAT with RT-PCR on handheld device for MTB	Molbio Diagnostics, Bigtec Labs	2013[54]	Comparative study NCT02252198 under way
TREK Sensititre MYCOTB MIC plate	Dry microdilution plate to detect MICs for FLD + SLD (except PZA)	TREK Diagnostic Systems, Thermo Fisher Scientific	2014[55]	
ANTIBODY/ANTIGEN DETECTION				
MBio Array System	POC cartridge to measure ~57 simultaneous MTB antigen-antibody reactions	MBio Diagnostics, FIND	2014[56]	

DST: drug-susceptibility testing
EMB: ethambutol
FLD: first-line drugs (INH, RIF, EMB, PZA)
FQ: fluoroquinolone
INH: isoniazid
MDR-TB: multidrug-resistant TB
MIC: minimum inhibitory concentration
MTB: *Mycobacterium tuberculosis*
MYCOTB: *Mycobacterium tuberculosis*
NAAT: nucleic-acid amplification test
POC: point of care
PZA: pyrazinamide
RIF: rifampin
RT-PCR: real-time polymerase chain reaction
SLD: second-line drug
SLID: second-line injectable drug (e.g., amikacin, capreomycin, or kanamycin)

It is clear from the paucity of published studies, that, as noted in the UNITAID landscape analysis, despite the potential of some of the newer portable, handheld NAATs' being made available closer to where people get diagnosis and treatment: "[a] significant deterrent to widespread application of NAATs is the need for appropriate field evaluation of newer tests. Currently there have been limited assessments of the next-generation NAATs, with only two evaluations of LoopAMP MTBC™ Detection Kit and EasyNAT™, and one each for Genedrive®, Truelab™ and FluoroCycler technologies. For most of these products, on the market for a few years now, more performance data are needed to inform NTP [national TB program] policies."[17]

The evidence base for most new TB diagnostic tests in the pipeline is shockingly weak for most of the so-called fast followers to the Xpert MTB/RIF test. It is distressing that neither the Hain GenoType MTBDR*sl* test nor Eiken's PURE-LAMP test has yet generated enough evidence to overcome the WHO expert panels' 2013 refusal to recommend these tests due to insufficient evidence.[15,16]

For Xpert, a pragmatic randomized trial conducted in South Africa and presented at the 2015 Conference on Retroviruses and Opportunistic Infections (CROI) showed that the immediate addition of Xpert had no impact on mortality versus standard of care (microscopy, with Xpert deferred). The investigators concluded: "a sensitive diagnostic test needs to be supported by systems linking to appropriate care, particularly ensuring that people know their HIV status and those eligible…start ART promptly."[21] Yet the impact of Xpert on earlier treatment initiation in many settings is undeniable.[21a]

On the more encouraging side, another paper presented at CROI 2015 introduced a new version of the test, the Xpert MTB/RIF Ultra, with sensitivity claimed comparable to culture.[37,38] Other planned improvements to the platform include adding common isoniazid resistance mutations and HIV RNA measurement.

Among people with HIV in a Ugandan study, the Alere Determine TB LAM – a simple urine dipstick that gives results in under 30 minutes – detected over half of those with culture-positive TB[22] and was "highly cost-effective compared with usage of either sputum smear-microscopy or Xpert alone."[23] Indeed, "[t]he sensitivity of the combination of Xpert and LF-LAM was 85% (88/103 95% CI 0.77–0.92), which was superior to either test alone ($P<0.05$) and approached sensitivity of sputum liquid culture testing (94%, 95% CI 0.88-0.98, $P=0.17$)."[24] The test is much less useful among people with higher CD4 counts, however. These results, and a substantial body of additional evidence,[24a] support a WHO recommendation for the use of the lateral flow LAM test, at least among HIV-positive people with low CD4 counts.

Future Directions

Madhukar Pai and Marco Schito write:

> The ongoing rollout of Xpert MTB/RIF has had a positive influence on the TB diagnostics landscape, has attracted new investments and product developers, and has created a robust pipeline of technologies… However, the Xpert technology was not designed to reach lower tiers of the healthcare system or to meet all needs ([e.g.,], it cannot detect latent *M. tuberculosis* infection or resistance against multiple drugs. Despite initiatives to reduce the price, high costs continues to be a hurdle….A recent survey of 22 countries with a high tuberculosis burden (HBCs) showed that, while a majority (86%) of these countries have a policy or algorithm for use of Xpert technology, current implementation is mostly donor funded, dependent largely on testing in centralized laboratories, and primarily involves patients with presumed drug-resistance or HIV infection [see ref. 25]….This suggests that wide-scale implementation of Xpert technology has mostly occurred in South Africa, while other HBCs continue to rely heavily on smear microscopy.[19]

In April 2014, the WHO convened a priority-setting group to develop target product profiles for the highest-priority consensus indications, which were:

- a biomarker test: "[a] point-of-care non-sputum-based test capable of detecting all forms of TB by identifying characteristic biomarkers or biosignatures…";
- a triage test: "[a] point-of-care triage test, which should be a simple, low-cost test that can be used by first-contact health-care providers to rule-out TB…";
- a smear-replacement test: "[a] point-of-care sputum-based test to be used as a replacement for smear microscopy…; and"
- a rapid DST test: "[a] rapid drug-susceptibility test that can be used at microscopy centers…."[26]

It's striking that this consensus group did not identify the need for a more definitive test for latent TB infection (LTBI) as a high priority as the current tests – tuberculin skin testing and IGRAs – have significant flaws, are not specific to MTB, and miss many cases; and in any case treatment of LTBI will be essential to eliminating new TB transmission.

In any case, with current scientific uncertainties and the continued likelihood of inadequate funding for TB R&D overall and for TB diagnostics research, these desiderata seem far away indeed. According to UNITAID:

> In the medium term, the need for a biomarker-based, low-cost, non-sputum-based test remains a key priority for TB diagnostics beyond the microscopy centre where the majority of people first seek care. Although biomarker discovery is an active area and several potential products (e.g. antigen or antibody detection tests; volatile organic compounds (VOCs); enzymatic detection) are under development, *no test under development is likely to be on the market with policy endorsements within the next three to five years* [emphasis added].[17]

With the exceptions of the urine LAM dipstick, the potential Xpert MTB/RIF Ultra, and GenoType MTBDR*sl* and PURE-LAMP – if stronger supporting evidence emerges – there are not a lot of test candidates likely to be reviewed and recommended by the WHO for use in middle- and low-income countries in the near future. The ideal POC biomarker test is clearly years off, and even the potential of VOCs remains remote unless programs have access to the 40 or so expertly trained giant African pouched rats, which can detect TB in sputum samples[43] – and it is unlikely that this innovative live diagnostic method could be scaled up any time soon.

Recommendations

1. **Invest in TB R&D and diagnostics research – including "R&D for new, biomarker-based triage/POC tests."**[57] The world needs to invest an additional US$270 million per year in TB diagnostics research, and US$2.0 billion annually for TB R&D to make this curable disease detectable and treatable for all.
2. **Integrate TB diagnostics research into ongoing treatment regimen studies**, and improve the integration of TB diagnostics and treatment research with implementation research in programmatic settings, including among people with HIV and children.
3. **Implement universal drug-susceptibility testing. "Push NTPs and health systems to think beyond sputum smears. Xpert is the quickest route to upfront DST. In parallel, build capacity for DST-guided MDR-TB therapy (so, capacity for liquid cultures)….We need next-generation DST ready for launch of new drug regimens."**[57] "Advocate for wider use of Xpert…among those with presumed TB, in children, people with HIV, and extrapulmonary TB."[57]

4. **"Eliminate inaccurate/misleading tests such as serology in China; restrict use of IGRAs for latent TB** (especially in India, SA, China)."[57]

5. **Increase screening and treatment for LTBI. "Demand systematic screening of contacts – especially children under 5 and people living with HIV."**[57]

6. **Improve the quality of research studies**, e.g., for follow-on NAA technologies, which have the potential to be cheaper, more portable, and more accessible than Xpert MTB/RIF but for which evidence of their effectiveness has been sorely lacking.

7. **Intensify investments in comparative studies** of new TB diagnostics and algorithms to optimize the use of current and emerging approaches in all important settings.

8. **Improve regulatory capacity to oversee TB diagnostics research** in all countries to ensure that NTPs, providers, and people with TB alike do not waste scarce resources on tests that lack specificity and sensitivity. The WHO has been right to set a high bar for recommending new TB diagnostics – and for recommending which tests *not* to use. Countries need to learn how to better evaluate existing tests with the same high standards. "Advocate for new tools to be rapidly evaluated for policy review."[57]

9. **Implement new TB diagnostic tests and algorithms in a coherent way** across health systems to enable diagnosis of TB as broadly as possible and break out of the deeply inadequate vertical microscopy-center model. Currently some sites equipped with Xpert refuse to use it because they lack MDR-TB treatments – not realizing that many if not most cases picked up by Xpert are simply smear-negative or extrapulmonary TB that is drug-sensitive. TB prevention, care, and treatment need to be integrated into health systems more broadly and effectively.

10. **Institute open access to all TB R&D publications.** Keeping research with results critical for the health of millions in resource-limited settings behind a firewall inhibits the free circulation of new scientific knowledge.

11. **Insist on universal access to and, where needed, uptake of all new evidence-based TB diagnostic tests without stock-outs, excessive prices, or arbitrary access barriers among different sectors of the health system, such as the current restriction of concessional Xpert pricing to public-sector programs.**

12. **Involve communities affected by TB, people living with TB, survivors of TB, and activists** in TB diagnostics research, implementation, rollout, and evaluation to improve community understanding and create greater demand for better solutions.

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REFERENCES

CROI: Conference on Retroviruses and Opportunistic Infections

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1. Benjamin W. "Zentralpark" [Central Park], Gesammelte Schriften [Collected Writings]. Vol. 1. Frankfurt am Main: Suhrkamp Verlag; 1974. p. 683. [translation by Mark Harrington]
2. World Health Organization. Global tuberculosis control report 2014. Geneva: World Health Organization; 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf.
3. Frick M. Figure 1: annual Global Plan research funding targets versus 2013 funding. In: 2014 report on tuberculosis research funding trends, 2005–2013. 2nd ed. New York: Treatment Action Group; 2015 May. p. 2. http://www.treatmentactiongroup.org/tbrd2014.
4. Stop TB Partnership/World Health Organization. The global plan to stop TB 2011–2015. Geneva: World Health Organization; 2010. http://www.stoptb.org/assets/documents/global/plan/tb_globalplantostoptb2011-2015.pdf.
5. Gandhi NR, Sturm AW, Pawinski R, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet. 2006 Nov 4;368(9547):1575–80. http://dx.doi.org/10.1016/S0140-6736(06)69573-1.
6. World Health Organization. Use of liquid TB culture and drug susceptibility testing (DST) in low and medium income settings. Summary report of the expert group meeting on the use of liquid culture media, Geneva, 26 March, 2007. Geneva: World Health Organization; 2007. http://www.who.int/tb/laboratory/use_of_liquid_tb_culture_summary_report.pdf.
7. World Health Organization. Policy statement. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Geneva: World Health Organization; 2008. http://www.who.int/tb/features_archive/policy_statement.pdf.
8. World Health Organization. Policy statement. Noncommercial culture and drug-susceptibility methods for screening persons at risk for multidrug-resistant tuberculosis. Geneva: World Health Organization; 2011. http://whqlibdoc.who.int/publications/2011/9789241501620_eng.pdf.
9. World Health Organization. Policy statement. Same-day diagnosis of tuberculosis by microscopy. Geneva: World Health Organization; 2011. http://apps.who.int/iris/bitstream/10665/44603/1/9789241501606_eng.pdf.
10. World Health Organization. Policy statement. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis. Geneva: World Health Organization; 2011. http://apps.who.int/iris/bitstream/10665/44602/1/9789241501613_eng.pdf.
11. World Health Organization. Policy statement. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva: World Health Organization; 2011. http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf.
12. World Health Organization. Policy update. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Geneva: World Health Organization; 2013. http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf.

12a. Detjen AK, DiNardo AR, Leyden J, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Resp Med. 2015 Jun;3(6):451–61. http://dx.doi.org/10.1016/S2213-2600(15)00095-8.

12b. Denkinger CM, Pai M. Using cerebrospinal fluid for the diagnosis of tuberculous meningitis with GeneXpert. Eur Respir J. 2014 Oct;44(4):1095–6. doi: 10.1183/09031936.00106914.

13. World Health Organization. Commercial serodiagnostic tests for diagnosis of tuberculosis: expert group meeting report, 22 July 2010. Geneva: World Health Organization; 2011. http://whqlibdoc.who.int/hq/2011/WHO_HTM_TB_2011.14_eng.pdf.
14. World Health Organization. Policy statement. Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries. Geneva: World Health Organization; 2011. http://www.who.int/tb/features_archive/policy_statement_igra_oct2011.pdf.
15. World Health Organization. The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis. Expert group meeting report. Geneva: May 2013. Geneva: World Health Organization; 2013. http://apps.who.int/iris/bitstream/10665/83142/1/WHO_HTM_TB_2013.05_eng.pdf.
16. World Health Organization. The use of molecular line probe assay for the detection of resistance to second-line anti-tuberculosis drugs. Expert group meeting report – Geneva: February 2013. Geneva: World Health Organization; 2013. http://apps.who.int/iris/bitstream/10665/78099/1/WHO_HTM_TB_2013.01.eng.pdf.
17. UNITAID. 2014 tuberculosis diagnostics technology and market landscape, 3rd edition. Geneva: World Health Organization; 2014. http://www.unitaid.eu/images/marketdynamics/publications/UNITAID_TB_Diagnostics_Landscape_3rd-edition.pdf.
18. Pai M. Innovations in tuberculosis diagnostics: progress and translational challenges. EbioMedicine. 2015 Jan 31;2(3):182–3. http://dx.doi.org/10.1016/j.ebiom.2015.01.018.
19. Pai M, Schito M. Tuberculosis diagnostics in 2015: landscape, priorities, needs, and prospects. J Infect Dis. 2015 Apr 1;211 Suppl 2:S21–8. doi: 10.1093/infdis/jiu803.
20. Dorman S. Advances in the diagnosis of tuberculosis: current status and future prospects. Int J Tuberc Lung Dis. 2015 May;19(5):504–16. http://dx.doi.org/10.5588/ijtld.15.0048.

21. Churchyard G, McCarthy K, Fielding KL, et al. Effect of Xpert MTB/RIF on early mortality in adults with suspected TB: a pragmatic randomized trial (Abstract 95). 22nd CROI; 2015 February 23–26. http://www.croiconference.org/sessions/effect-xpert-mtbrif-early-mortality-adults-suspected-tb-pragmatic-randomized-trial.

21a. Theron G, Zijenah L, Chanda D, 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. 2014 Feb 1;383(9915):424–35. http://dx.doi.org/10.1016/S0140-6736(13)62073-5.

22. Nakiyingi L, Moodley VM, Manabe YC, et al. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. J Acquir Immune Defic Syndr. 2014 Jul 1;66(3):270–9. doi: 10.1097/QAI.0000000000000151.

23. Shah M, Dowdy D, Joloba M, et al. Cost-effectiveness of novel algorithms for rapid diagnosis of tuberculosis in HIV-infected individuals in Uganda. AIDS. 2013 Nov 28:27(18:)2883–92. doi: 10.1097//QAD.0000000000000008.

24. Shah M, Ssengooba W, Armstrong D, et al. Comparative performance of urinary lipoarabinomannan assays and Xpert MTB/RIF in HIV-infected individuals with suspected tuberculosis in Uganda. AIDS. 2014 June 1;28(9):1307–14. doi: 10.1097/QAD.0000000000000008.

24a. 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 Apr 26;12:103. doi: 10.1186/1471-2334-12-103. Review.

25. Qin ZZ, Pai M, Van Gemert W, et al. How is Xpert MTB/RIF being implemented in 22 high tuberculosis burden countries? Eur Respir J. 2015 Feb;45(2):549–54. doi: 10.1183/09031936.00147714.

26. Denkinger C, Kik S, Casenghi M. Meeting report. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. 28–29 April 2014, Geneva, Switzerland. Geneva: World Health Organization; 2014. http://apps.who.int/iris/bitstream/10665/135617/1/WHO_HTM_TB_2014.18_eng.pdf.

27. Op den Buijs IOM, Seagar AL, Baltar P, et al. Development and evaluation of the detection of Mycobacterium tuberculosis complex on the BD MAX™ system in a European multicenter study. Poster session presented at: European Congress of Clinical Microbiology and Infectious Disease, 2014 May 10–13; Barcelona, Spain. http://www.pamm.nl/fileadmin/media-archive/corporate-new/Bestanden/Over_PAMM/Publicaties/ECCMID_2014_MTB_Final_JB.pdf.

28. Mhimbara FA, Bholla M, Sasamalo M, et al. Detection of Mycobacterium tuberculosis by EasyNAT diagnostic kit in sputum samples from Tanzania. J Clin Microbiol. 2015 Apr;53(4):1342–4. doi: 10.1128/JCM.03037-14.

29. Bwanga F, Disqué C, Lorenz MG, et al. Higher blood volumes improve the sensitivity of direct PCR diagnosis of blood stream TB among HIV-positive patients: an observational study. BMC Infect Dis. 2015 Feb 6;15:48. doi: 10.1186/s12879-015-0785-3.

30. González Mediero G, Vázquez Gallardo R, Pérez Del Molino ML, et al. Evaluation of two commercial nucleic acid amplification kits for detecting Mycobacterium tuberculosis in saliva samples. Oral Dis. 2015 May;21(4):451–5. doi: 10.1111/odi.12302.

31. Zhu L, Liu A, Martinez L, et al. Diagnostic value of GeneChip for detection of resistant Mycobacterium tuberculosis in patients with differing treatment histories. J Clin Microbiol. 2015 Jan;53(1):131–5. doi:10.1128/JCM.02283-14.

32. At the 2015 Joint Partners Forum for Strengthening and Aligning TB Diagnosis and Treatment (WHO, Geneva, April 27–30, 2015) Stakeholders Roundtable on DST Diagnostic Technologies, Hain stated that "GenoType MDRTBsl ver 2.0 was now available with 'increased sensitivity for kanamycin and fluoroquinolones.'" New data were not presented. www.stoptb.org/wg/gli/assets/documents/M7/4.%20HAIN.pdf.

33. Theron G, Peter J, Richardson M, et al. The diagnostic accuracy of the GenoType® MTBDRsl assay for the detection of resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev. 2014 Oct 29:10:CD010705. doi: 10.1002/14651858.CD010705.pub2. The review included 21 studies (14 on direct sputum, 5 on culture, 2 on both). Eleven studies were in low- and middle-income countries. The authors concluded: "In adults with TB, a positive MTBDRsl result for FQ resistance, SLID resistance, or XDR-TB can be treated with confidence. However, MTBDRsl does not detect approximately one in five cases of FQ-resistant TB, and does not detect approximately one in four cases of SLID-resistant TB. Of the three SLIDs, MTBDRsl has the poorest sensitivity for kanamycin resistance. MTBDRsl will miss between one in four and one in three cases of XDR-TB. The diagnostic accuracy of MTBDRsl is similar when done using either culture isolates or smear-positive sputum. As the location of the resistance causing mutations can vary on a strain-by-strain basis, further research is required on test accuracy in different settings and, if genetic sequencing is used as a reference standard, it should examine all resistance-determining regions. Given the confidence one can have in a positive result, and the ability of the test to provide results within a matter of days, MTBDRsl may be used as an initial test for second-line drug resistance. However, when the test reports a negative result, clinicians may still wish to carry out conventional testing." http://onlinelibrary.wiley.com/enhanced/doi/10.1002/14651858.CD010705.pub2.

34. Rienthong S, Boonin C, Chaiyasirinrote B, et al. Evaluation of a novel line-probe assay for genotyping-based diagnosis of Mycobacterium tuberculosis in Thailand. Int J Tuberc Lung Dis. 2015 Jul 19;(7):817–22. doi: 10.5588/ijtld.14.0311.

35. Hu S, Li G, Li H, et al. Rapid detection of isoniazid resistance in Mycobacterium tuberculosis isolates by use of real-time-PCR-based melting curve analysis. J Clin Microbiol. 2014 May;52(5):1644–52. doi: 10.1128/JCM.03395-13.

36. Zhang T, Hu S, Li G, et al. Evaluation of the MeltPro TB/STR assay for rapid detection of streptomycin resistance in Mycobacterium tuberculosis. Tuberculosis (Edinb). 2015 Mar;95(2):162–9. doi: 10.1016/j.tube.2014.12.004.

37. Abbott (Press Release). Abbott introduces new molecular test to aid in the diagnosis of tuberculosis. 2014 October 8. https://www.abbottmolecular.com/aboutus/press-releases/abbott-introduces-new-molecular-test-to-aid-in-diagnosis-of-tuberculosis.html.

38. Chen JH, She KK, Kwong TC, et al. Performance of the new automated Abbott RealTime MTB assay for rapid detection of Mycobacterium tuberculosis complex in respiratory specimens. Eur J Clin Microbiol Infect Dis. 2015 June 13. doi: 10.1007/s10096-015-2419-5. [Epub ahead of print.]

39. Lee YS, Kang MR, Jung H, et al. Performance of REBA MTB-XDR to detect XDR TB in an intermediate-burden country. J Infect Chemother. 2015 May;21(5):346–51. doi: 10.1016/j.jiac.2014.12.009.

40. "For the Xpert MTB/RIF Ultra (Ultra) assay, we developed a new sample processing cartridge that doubled the amount of purified DNA delivered to the PCR reaction. Four newly designed probes that detected mutations in rpoB gene replaced the five Xpert real-time probes. Real-time Mtb detecting probes targeting IS6110 and IS1081 were added. Cartridge fluidics and PCR cycling were optimized. Assay LODs were tested by spiking Mtb H37Rv and BCG cells into sputum samples, treating with Sample Reagent, splitting samples, and testing with Xpert and Ultra. RIF-R detection was tested with a panel of 30 different RIF-R Mtb rpoB mutants. LOD was defined as the lowest CFU that could be detected in at least 19/20 (95%) tests. Results. Ultra was significantly more sensitive than Xpert. In sputum samples spiked with Mtb H37Rv, Ultra had an LOD of 5 CFU/ml compared to an LOD of 50 CFU/ml for Xpert (p=0.001). In sputum samples spiked with BCG, Ultra had an LOD of 25 CFU/ml compared to an LOD of 165 CFU/ml for Xpert (p=0.01). Ultra detected 30 different RIF-R Mtb rpoB mutants as RIF-R (sensitivity 100%). None of the 25 RIF-S rpoB wild type samples and none of the 3 RIF-S synonymous rpoB Q513Q (1) and F514F (2) mutant samples were detected as RIF-R (specificity 100%). Ease of use was identical for Xpert and Ultra. Conclusions. The new Ultra assay is much more sensitive than Xpert, and is likely to be as sensitive as liquid TB culture. Ultra detects RIF-R as efficiently as Xpert; but the specificity of Ultra RIF-R is likely to be higher due to improvements in assay design. The Ultra assay should significantly increase TB detection in smear-negative patients and provide more reliable RIF-R detection." Alland D, Rowneki M, Smith L. Xpert MTB/RIF Ultra: a new near-patient TB test with sensitivity equal to culture (Abstract 91). Paper presented at: 22nd CROI; 2015 February 23–26; Seattle, WA. http://www.croiconference.org/sessions/xpert-mtbrif-ultra-new-near-patient-tb-test-sensitivity-equal-culture.

41. Getahun H. Twitter post. 2015 February 25, 2:05 p.m. https://twitter.com/haileygetahun/status/570660950853881856.

42. Weetjens BJ, Mgode GF, Machang'u RS, et al. African pouched rats for the detection of pulmonary tuberculosis in sputum samples. Int J Tuberc Lung Dis. 2009 Jun;13(6):737-43. http://www.ingentaconnect.com/content/iuatld/ijtld/2009/00000013/00000006/art00010.

43. Mgode GF, Cohen-Bacrie S, Bedotto M, et al. Mycobacterium genotypes in pulmonary tuberculosis infections and their detection by trained African giant pouched rats. Curr Microbiol. 2015 Feb;70(2):212–8. doi: 10.1007/s00284-014-0705-6.

44. Breuninger M, van Ginneken B, Philipsen RH, et al. Diagnostic accuracy of computer-aided detection of pulmonary tuberculosis in chest radiographs: a validation study from sub-Saharan Africa. PLoS One. 2014 Sep 5;9(9):e106381. doi: 10.1371/journal.pone.0106381.

45. Muyoyeta M, Moyo M, Kasese N, et al. Implementation research to inform the use of Xpert MTB/RIF in primary health care facilities in high TB and HIV settings in resource constrained settings. PLoS ONE. 2015 Jun 1;10(6):e0126376. doi :10.1371/journal. pone.0126376.

46. Hogeweg L, Sanchez CI, Maduskar P, et al. Automatic detection of tuberculosis in chest radiographs using a combination of textural, focal, and shape abnormality analysis. IEEE Transactions on Medical Imaging. 2015 Feb 19. doi: 10.1109/TMI.2015.2405761. [Epub ahead of print]

47. Castan P, de Pablo A, Fernández-Romero J, et al. Point-of-care system for detection of Mycobacterium tuberculosis and rifampin resistance in sputum samples. J Clin Microbiol. 2014 Feb;52(2):502–7. http://www.ncbi.nlm.nih.gov/pubmed/25634305.

48. Rice JE, Reis AH Jr, Rice LM, et al. Fluorescent signatures for variable DNA sequences. Nucleic Acids Res. 2012 Nov;40(2):e164. doi:10.1093/nar/gks731.

49. Mitarai S, Kato S, Ogata H. Comprehensive multicenter evaluation of a new line probe assay kit for Identification of mycobacterium species and detection of drug-resistant Mycobacterium tuberculosis. J Clin Microbiol. 2012 Mar;50(3):884–90. doi: 10.1128/JCM.05638-11. Last year's Pipeline Report referenced a 2013 article in Japanese. We have been unable to review the entire article. The abstract is quite vague. "We found that LiPA enabled the rapid identification of M. tuberculosis, M. avium, M. intracellulare, and M. kansasii. When the results of the LiPA and conventional drug susceptibility tests were compared, there was no difference in the susceptibility to rifampicin, pyrazinamide, and levofloxacin; however, there was a difference in the susceptibility to isoniazid." Matsumoto T, Ogata H, Toyota E, et al. Clinical evaluation of a line probe assay kit for the identification of Mycobacterium species and detection of drug- resistant Mycobacterium tuberculosis. [Article in Japanese]. Kekkaku. 2013 Mar;88(3):291–6.

50. Ou X, Li Q, Xia H, et al. Diagnostic accuracy of the PURE-LAMP test for pulmonary tuberculosis at the county-level laboratory in China. PLoS One. 2014 May 1;9(5):e94544. doi: 10.1371/journal.pone.0094544.

51. Cho E, Chola Shamputa I, Kwak H-K, et al. Utility of the REBA MTB-Rifa® assay for rapid detection of rifampicin resistant Mycobacterium tuberculosis. BMC Infect Dis.. 2013 Oct 13:478. doi:10.1186/1471- 2334-13-478.

52. Tanaka H, Hirose H, Kato Y, et al. Clinical evaluation of TRC Rapid M.TB for detection of Mycobacterium tuberculosis complex in respiratory and nonrespiratory specimens. J Clin Microbiol. 2010 May;48(5):1536-41. doi:10.1128/JCM.01758-09.

53. Tosoh Corporation (Japan). Annual report 2014: research and development. [place unknown]: Tosoh Corporation; 2015. p 2. www.tosoh.com/investors/annual-reports/2014.

54. Nikam C, Jagannath M, Narayanan M, et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. PloS One. 2013;8(1):e51121. doi: 10.1371/journal.pone.0051121.

55. Lee J, Armstrong D, Ssengooba W, et al. Sensititre MYCOTB MIC plate for testing Mycobacterium tuberculosis susceptibility to first- and second-line drugs. Antimicrob Agents Chemother. 2014 Jan;58(1):11–18. doi: 10.1128/AAC.01209-13.

56. Greef C, Husar G, Gray C, et al. Highly multiplexed detection of antibodies in whole blood during tuberculosis infection (Abstract 813). 21st CROI; 2014 March 3–6; Boston, MA. https://www.iasusa.org/sites/default/files/tam/22-e1-4.pdf.

57. Pai M. TB diagnostics in 2015: landscape and opportunities for advocacy. PowerPoint presentation to Global TB community advisory board; 2015 May 20; New York, NY.

58. Batz H-G, Cook GS, Reid SD. Towards lab-free tuberculosis diagnosis. London: Treatment Action Group, Stop TB Partnership Global TB/HIV Working Group, Imperial College, MSF Access to Essential Medicines Campaign; 2011 August. http://www.msfaccess.org/sites/default/files/MSF_assets/TB/Docs/TB_Report_TowardsLabFreeTBDX_2011_ENG.pdf.