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Editorial

Advances in TB diagnostics: A critical element for the elimination toolkit

Despite being preventable and usually curable, over 10 million people worldwide fell ill with tuberculosis (TB) in 2022, and an estimated 1.3 million deaths were caused by TB[1]. Till date, the diagnosis remains the largest gap in the cascade of TB care. Approximately one in three people estimated to develop TB remained undiagnosed in the year 2022, and for those that were eventually diagnosed, only about 60 per cent had a bacteriological confirmation[1]. Drug-resistant TB (DR-TB) is an even more challenge to diagnose, with only two in five of the 0.4 million global cases being identified as resistant and started on appropriate treatment. There also remain large delays before diagnosis and treatment, contributing to individual morbidity and mortality, including post-TB lung disease, ongoing transmission and economic costs for TB patients and their households.

The End TB strategy aims to significantly reduce the incidence of TB, deaths and the costs faced by patients as well as their households over the next 10 years[2]. Early diagnosis of TB (including drug resistance) is the key component of the End TB strategy as well as the TB elimination toolkit. Currently, the most widely used microbiological diagnostic test for TB is still sputum smear microscopy, despite its poor sensitivity, operational challenges, and World Health Organization (WHO) recommendations for rapid molecular tests[1,3]. The global targets set out at the 2023 second UN high-level meeting on TB (*https://www.who.int/activities/preparing-for-the-un-high-level-meeting-on-the-fight-against-tuberculosis--2023*) explicitly aim for 100 per cent coverage of rapid diagnostic testing for TB by 2027.

To address the elimination of TB as a global public health problem, a wide range of technologies and tools in the field of TB diagnostics will be needed. This was recognized by the WHO, and to help marry innovation with the needs of end-users and healthcare systems, they released a range of Target Product Profiles (TPPs) in 2014 (currently being updated)[4]. The TB diagnostics pipeline has grown considerably in recent years in terms of the number of products, tests, and technologies under development[5]. This pipeline has also been significantly bolstered by the COVID-19 pandemic, which accelerated research into diagnostic technologies that could be applied to TB[6]. Despite this, no novel TB test currently meets all the TPP targets.

Currently available rapid molecular tests

The WHO first recommended an initial rapid molecular diagnostic test for TB in 2010. The Xpert MTB/RIF assay (Cepheid, USA) is a low-complexity, semi-automated, cartridge-based molecular test that is suitable for implementation in high-burden settings without needing containment level-3 molecular pathology laboratories[7]. This assay was modified to increase sensitivity (through additional targets) in 2017, and the Xpert MTB/RIF Ultra is now widely implemented with a global access price of US $8 per cartridge[8]. However, Xpert is not truly point-of-care, as it requires a reliable power supply, a favourable operating environment and frequent maintenance. The Truenat platform (Molbio, India) is a more recently WHO-recommended, low-complexity, chip-based real-time PCR assay[9]. Due to this platform being battery-operated, it is more suitable for point-of-care settings. Both Truenat and Xpert assays can also detect >90 per cent of mutations associated with rifampicin (RIF) resistance.

Despite the gradual rollout of these and other more centralized molecular tests for TB over the last decade, most (38% in 2021 and 47% in 2022) patients do not have access to these diagnostics[1]. There is a real need for more accessible and truly point-of-care molecular assays with high sensitivities. Both near-patient and instrument-free point-of-care TB molecular tests are in development and will require parallel market interventions to ensure quick adoption to meet global End TB targets.

Alternative sampling and testing approaches: Moving away from sputum

Sputum is still the most relied on sample type for TB testing, but it is challenging to collect and process, and therefore, contributes to the large diagnostic gap for TB. Many people with presumed TB are unable to produce adequate sputum samples for testing, especially children, people living with HIV and those with the extra-pulmonary disease. Recent innovations in TB diagnostics have focussed on more accessible and useable, non-sputum-based sample types.

The lipoarabinomannan (LAM) antigen is a major cell wall constituent of MTB and was discovered in the urine of individuals with TB in the 1990s[10]. The Determine™ TB LAM Ag test (Abbott, USA) lateral flow assay uses only 60 µL of urine and takes 20 minutes for TB detection. It was found to be useful for TB diagnosis in people living with HIV, and despite moderate overall sensitivity, it has been recommended for TB diagnosis by WHO in this population. Several developers are working on the next-generation urine antigen tests to improve sensitivity with higher affinity antibodies and different urine concentration methods. These tests offer promise to meet WHO TPP targets in all patient groups (including those not living with HIV), provided the variability in detection around the cut-off, as first described for SILVAMP TB LAM (Fujifilm, Japan), is resolved[11].

Oral swabs became a mainstay sample during the COVID-19 pandemic and have also emerged as a potential alternative sample type to sputum for TB. When coupled with rapid molecular tests such as Xpert Ultra and Truenat, the sensitivity of tongue swabs for TB can be as high as 70-75 per cent compared to sputum[12]. Performance appears to be linked to swab type, collection, storage, and the pairing with the appropriate back-end molecular test (with tests that are able to detect free-DNA being more preferable), therefore, further optimization may well improve the sensitivity[13]. Furthermore, current studies compare swabs to sputum-based testing, whereas a major benefit is likely to be an increase in the yield of microbiological diagnoses of TB in those unable to produce adequate sputum samples, for example, in community-based TB screening programmes.

Breath is the ultimate non-invasive sample for TB diagnosis with a potential link to transmission in addition to the diagnostic use-case[14]. Several volatile organic compounds (VOC)-based tests assays from exhaled breath have been evaluated for TB, from electronic noses to Giant African Pouched Rats, with sensitivities ranging from 60-90 per cent[15]. The lack of specificity and variable performance hinder current assays for TB. Nevertheless, the promise of VOC detection from breath is shown by commercialized tests for SARS-CoV-2[16]. Direct detection of MTB in breath is also feasible. The respiratory aerosol sampling chamber (RASC) demonstrates the proof-of-principle with high specificity. However, the main challenge remains the low abundance of MTB in breath[17]. More realistic point-of-care solutions coupled with MTB detection by molecular assays are being explored, such as facemask sampling[18]. Other non-sputum sampling being evaluated includes molecular detection of TB from stool and blood sampling for host-response transcriptional signatures[19,20].

Digital health technologies and artificial intelligence (AI) also offer opportunities for diagnostic solutions for TB. Computer-aided detection (CAD) softwares are being used to automate reading and interpreting digital chest X-rays, although implementation challenges remain[21]. Other AI-based tools to classify lung and cough sounds are being explored for TB screening[22].

Drug-resistant TB

The current diagnosis of DR-TB requires several steps and is challenging even in well-resourced settings. WHO recommends that all bacteriologically confirmed TB undergo drug susceptibility testing (DST) for commonly used anti-TB drugs[23]. First-line molecular assays provide sensitive detection of RIF resistance, but testing for isoniazid and fluoroquinolone resistance is increasingly important. Traditional culture-based phenotypic DST are highly complex, labour-intensive, have long turnaround time and not accessible for most people at risk of DR-TB.

Increased understanding of the molecular level mechanisms of drug resistance over the past decade is leading to advances in the diagnosis of DR-TB. Several rapid molecular platforms now provide DST results for RIF and isoniazid, and two assays have been recommended by WHO for fluoroquinolone resistance (Xpert MTB/XDR [Cepheid, USA], and Genotype MTBDR*sl* [Bruker/HainLifescience, Germany])[24]. However, more comprehensive rapid molecular diagnostics to accurately detect first- and second-line drug resistance as well as better point-of-care solutions are needed to close the diagnostic gap for DR-TB.

Next-generation DNA sequencing (NGS) has also facilitated the management of DR-TB. WHO has collated almost 200 drug-resistance mutations in its catalogue of mutations[25]. However, current workflows require culture of MTB prior to NGS, and therefore its use has been constrained by high cost and turnaround time. Targeted NGS approaches straight from patients' specimens hold the most promise, with research ongoing to amplify MTB genomes to improve sensitivity and the possibility of all processing and workflow on a single platform. Recent WHO guidelines recommend targeted NGS and are now supported by an operational handbook[26]. One potential limitation is the confidence of resistance gene targets for newer DR-TB drugs for example, bedaquiline, pretomanid and linezolid.

Conclusions and the way forward

Ending TB requires accessible, affordable and accurate diagnostics to be coupled with better treatment strategies. Technical innovations present the possibility of a range of diagnostic solutions to allow this. We envisage a future where people with presumed TB can provide a range of samples (sputum, swabs, urine, blood) to be tested for TB with rapid diagnostics at the point of care, including the rapid detection of DR-TB to allow for early initiation of an effective TB treatment regimen. This will not only require technology innovation but also political will and considerable investment to translate promising innovations into implementable diagnostic interventions that reach the 'missing millions' of people with undiagnosed TB.

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References

1. World Health Organization. *Global Tuberculosis Report 2023*. Available from: *https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023*, accessed on April 4, 2024.
2. World Health Organization. *The End TB Strategy*. Available from: *https://cdn.who.int/media/docs/default-source/documents/tuberculosis/end-tb-strategy-information-sheet8817f818-feaa-49ac-b26a-92c5b9dca034.pdf?sfvrsn=d6235a67_1&download=true,* accessed on April 4, 2024.
3. World Health Organization. *WHO consolidated guidelines on tuberculosis*. Available from: *https://www.who.int/publications-detail-redirect/9789240029415*, accessed on April 4, 2024.
4. Kik SV, Denkinger CM, Casenghi M, Vadnais C, Pai M. Tuberculosis diagnostics: Which target product profiles should be prioritised? *Eur Respir J* 2014; *44* : 537–40.
5. Treatment Action Group. Pipeline Report 2023. *Tuberculosis Diagnostics*. Available from: *https://www.treatmentactiongroup.org/wp-content/uploads/2023/11/2023_pipeline_TB_diagnostics_final.pdf*, accessed on April 4, 2024.
6. Yerlikaya S, Broger T, Isaacs C, Bell D, Holtgrewe L, Gupta-Wright A, *et al.* Blazing the trail for innovative tuberculosis diagnostics. *Infection* 2024; *52* : 29-42.
7 Lawn SD, Nicol MP. Xpert® MTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol* 2011; *6* : 1067-82.
8. The Global Fund. *New Pricing for Cepheid GeneXpert Tuberculosis Testing*. Available from: *https://www.theglobalfund.org/media/13442/operational_2023-10-cepheid-genexpert-tb-testing_briefingnote_en.pdf*, accessed on April 4, 2024.
9. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, *et al.* Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. *PloS One* 2013; *8* : e51121.
10. 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.
11. Huerga H, Bastard M, Lubega AV, Akinyi M, Antabak NT, Ohler L, *et al.* Novel Fuji LAM assay to detect tuberculosis in HIV-positive ambulatory patients in four African countries: a diagnostic accuracy study. *Lancet Glob Health* 2023; *11* : e126-35.
12. Wood RC, Luabeya AK, Dragovich RB, Olson AM, Lochner KA, Weigel KM, *et al.* Diagnostic accuracy of tongue swab

testing on two automated tuberculosis diagnostic platforms, Cepheid Xpert MTB/RIF Ultra and Molbio Truenat MTB Ultima. *J Clin Microbiol* 2024 : e0001924.

13. Luabeya AK, Wood RC, Shenje J, Filander E, Ontong C, Mabwe S, *et al.* Noninvasive detection of tuberculosis by Oral Swab Analysis. *J Clin Microbiol* 2019; *57* : e01847-18.
14. Saktiawati AMI, Putera DD, Setyawan A, Mahendradhata Y, van der Werf TS. Diagnosis of tuberculosis through breath test: a systematic review. *Ebio Medicine* 2019; *46* : 202-14.
15. Mulder C, Mgode GF, Ellis H, Valverde E, Beyene N, Cox C, *et al.* Accuracy of giant African pouched rats for diagnosing tuberculosis: comparison with culture and Xpert® MTB/RIF. *Int J Tuberc Lung Dis* 2017; *21* : 1127-33.
16. Myers R, Ruszkiewicz DM, Meister A, Bartolomeu C, Atkar-Khattra S, Thomas CLP, *et al.* Breath testing for SARS-CoV-2 infection. *Ebio Medicine* 2023; *92* : 104584.
17. Dinkele R, Gessner S, McKerry A, Leonard B, Seldon R, Koch AS, *et al.* Capture and visualization of live Mycobacterium tuberculosis bacilli from tuberculosis patient bioaerosols. *PLoS Pathog* 2021; *17* : e1009262.
18. Williams CM, Abdulwhhab M, Birring SS, De Kock E, Garton NJ, Townsend E, *et al.* Exhaled Mycobacterium tuberculosis output and detection of subclinical disease by face-mask sampling: prospective observational studies. *Lancet Infect Dis* 2020; *20* : 607-17.
19. Kay A, Vasiliu A, Carratala-Castro L, Mtafya B, Mendez Reyes JE, Maphalala N, *et al.* Performance of a stool-based quantitative PCR assay for the diagnosis of tuberculosis in adolescents and adults: a multinational, prospective diagnostic accuracy study. *Lancet Microbe* 2024 : e433-41.
20. Gupta-Wright A, Ha H, Abdulgadar S, Crowder R, Emmanuel J, Mukwatamundu J, *et al.* Evaluation of the xpert MTB host response assay for the triage of patients with presumed pulmonary tuberculosis: a prospective diagnostic accuracy study in Vietnam, India, the Philippines, Uganda, and South Africa. *Lancet Glob Health* 2024; *12* : e226-34.
21. Geric C, Qin ZZ, Denkinger CM, Kik SV, Marais B, Anjos A, *et al.* The rise of artificial intelligence reading of chest X-rays for enhanced TB diagnosis and elimination. *Int J Tuberc Lung Dis* 2023; *27* : 3 67-72.
22. Zimmer AJ, Ugarte-Gil C, Pathri R, Dewan P, Jaganath D, Cattamanchi A, *et al.* Making cough count in tuberculosis care. *Commun Med (Lond)* 2022; *2* : 83.
23. World Health Organization. *WHO operational handbook on tuberculosis*. Available from: *https://www.who.int/publications/i/item/9789240006997*, accessed on December 1, 2022.
24. World Health Organization. *Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis*. Available from: *https://www.who.int/publications-detail-redirect/9789240042575*, accessed on April 4, 2024.
25. World Health Organization. *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance*. Available from: *https://iris.who.int/bitstream/handle/10665/341981/9789240028173-eng.pdf?sequence=1*, accessed on April 4, 2024.
26. World Health Organization. *WHO consolidated guidelines on tuberculosis: Module 3: Diagnosis: Rapid diagnostics for tuberculosis detection*, 3rd ed. Available from: *https://www.who.int/publications-detail-redirect/9789240089488,* accessed on April 4, 2024.