RESEARCH ARTICLE

Advances in the laboratory diagnosis of tuberculosis.

Authors

Guadalupe García Elorraiga, PhD, Guillermo del Rey Pineda, PhD

Affiliations

[1]National Medical Center La Raza, CMNR, Mexican Social Security Institute, IMSS, Mexico City, Mexico
[2]Federico Gomez Children's Hospital, Mexico City, Mexico

Correspondence
Guadalupe García Elorraiga
Email: gelorriaga@webtelmex.net.mx

Abstract

Despite the advances made in the past 30 years in the laboratory diagnosis of tuberculosis (TB), only a small portion of the overall world population has benefitted. The World Health Organization (WHO) has recommended the use of nucleic acid amplification tests (NAAT) to detect TB instead of smear microscopy, since they can detect TB with greater precision, particularly in patients with paucibacillary disease and in individuals living with HIV. A broad range of molecular TB detection tests are currently being developed and evaluated, some for use in reference laboratories and others for peripheral medical care settings and point-of-care. There has been a surge of molecular tests designed, manufactured, and implemented in countries with a high TB load, and some are specifically meant for use in locations that are close to the patient. In terms of drug susceptibility testing, NAAT and next-generation sequencing may provide faster results than traditional phenotype culture. Further, the results of tests that detect or quantify cytokines released in the inflammatory process in latent tuberculosis infection (LTBI), such as the Interferon-Gamma Release Assay (IGRA), or that quantify IL-6 or other cytokines, depend, as in the tuberculin skin tests (TST), on the prevalence of TB in the tested population. We herein review the recent advances in TB detection tests and resistance to anti-TB drugs.

Keywords: Bacteriological diagnosis; Immunological diagnosis; Molecular diagnosis.

1. Introduction

Despite many advances and the continued fight against tuberculosis (TB), 7.1 million individuals worldwide were reported and diagnosed with TB in 2019,[1] 10 million individuals developed TB in 2018, and 1.5 million infections resulted in death. Many of these deaths could have been avoided if patients had timely access to diagnostic methods and treatment, but approximately one-third of TB cases are unable to obtain an accurate diagnosis. In low- and median-income countries (LMICs), the diagnosis of TB is mainly established by stained sputum smear microscopy in cases in which the disease is suspected; however, bacilloscopy can only detect 50 – 60% of all cases (positive acid-fast bacilli [AFB]). New methods with greater sensitivity in the diagnosis of TB, and that also detect drug-resistance, have recently become available but they are more costly.[2, 3] In the last decade, new molecular tests known as nucleic acid amplification tests (NAAT), were developed; they are based on the amplification of the target gene region of the *Mycobacterium tuberculosis* complex (MTBC), typically by polymerase chain reaction (PCR). The stage of latent tuberculosis infection (LTBI) is the point in which immune mediators are of key relevance as diagnostic tests, whereby the detection of disease is most helpful in decreasing the rates of anti-TB drug resistance and the development of drug toxicity due to the unwarranted administration of prophylactic therapy; further, biological therapies are associated with an increased risk of LTBI reactivation that may be prevented from progressing to TB with an appropriate diagnosis. This review will discuss advances in these tests in order to promote an avant-garde protocol for the rapid and routine TB diagnosis.

2. Bacteriological diagnosis

2.1 Specimen collection

In pulmonary TB (PTB), patient infectivity still hinges on the demonstration of AFB in sputum due to its simplicity and sensitivity, as well as the lack of the necessary technology to detect bacilli in the air, that is, infectious aerosols. A new cough aerosol sampling system (CASS) used in patients with proven PTB by culture and a positive smear in Uganda and Brazil, detected aerosols that could be cultured in 43% of patients. By polymerase chain reaction (PCR), molecular signals have been identified in the exhaled breath, at higher concentrations than in colony-forming units in solid cultures. A group in the United Kingdom has developed an innovative mask method to collect aerosolized *Mycobacterium tuberculosis* (MTB) in a modified facial mask. Also, high MTB DNA concentrations have been found in the exhaled breath of TB patients.[4]

2.2 Methods to decontaminate samples

The modified Petroff method is standard when decontaminating samples and the solid Löwenstein Jensen culture medium is used for inoculation. However, this method requires several steps and longer periods to execute, the use of a refrigerated centrifuge and a biosafety cabinet. The high demands and costs of a large technical infrastructure, and trained personnel, as well as the health system budget limitations in emerging countries, may preclude the performance of this test. Therefore, the development and/or use of lower-cost methods with the same sensitivity to decontaminate samples for subsequent inoculation, would increase the possibility of performing cultures in routine laboratories in different regions and hence, complete diagnostic TB coverage. The Ogawa-Kudoh method simplifies

decontamination, it takes 3 to 4 minutes, does not require centrifugation nor a biosecurity cabinet; it is, therefore, more economical. To decontaminate samples with the Ogawa-Kudoh method, a cotton swab should be inserted into the sputum sample´s most purulent portion, and then submerged in a 4% sodium hydroxide (NaOH) solution for 2 minutes. Remove the 4% NaOH excess by compression with a cotton swab against the vial´s wall, and then inoculate it in Ogawa-Kudoh medium (a slightly acidified medium, pH 6.4). This medium is highly sensitive and specific.[5]

2.3 Culture

Culture of *Mycobacterium tuberculosis* (MTB) remains the gold standard for the laboratory diagnosis of PTB. The Löwenstein Jensen (LJ) medium is recommended for culture and differentiation of MTB, but it is costlier than a bacilloscopy, colony development may take 2 to 8 weeks, and laboratories in low-income countries generally lack access to this test.

Another alternative is an assay by microscopic observation drug susceptibility (MODS), which has been recognized as a valid method to detect TB by the World Health Organization (WHO). It has greater sensitivity, it is faster and less costly than LJ culture. Indeed, extending the use of real-time PCR has a higher cost than direct microscopic examination ($2 for microscopy vs. $19.56 for PCR) but is probably more cost-effective when treatment costs are included (412 for microscopy vs. 382 for PCR) and yields a clear performance superiority.[6] Briefly, MODS requires a minimum Biosafety Level of 2. In MODS plates with 24 wells, place the negative control (medium with no sample) and the positive control preferably using the MTB H37Rv strain. The sputum samples must be digested and decontaminated by standard methods. Add 100 μL of 7H9 medium to each well and 900 μL of the sample suspension. Close the plate, place it in a Ziplock bag, and incubate at 37 ^{0}C. On incubation day 5, observe in an inverted light microscope at 10X. On days 5-9, MTB growth appears as small curves, commas, or spirals. Usually, colony formation progresses into cords.[7] If not observed, continue reading until day 21. This method could also be very useful in cases of extra-pulmonary TB (ETB), using sterile samples such as cerebrospinal, peritoneal, or pleural fluid, etc.

Indeed, extending the use of real-time PCR has a higher cost than direct microscopic examination (0.57$ for microscopy after Ziehl–Neelsen staining vs. 19.56$ for PCR) but is probably more cost-effective when treatment costs are included (412 for microscopy after Ziehl–Neelsen staining vs. 382 for PCR) and yields a clear performance superiority.

2.4 Blood culture in HIV

MTB Bloodstream infection (MTB BSI) is a frequent presentation of TB and it is potentially lethal in ambits with high HIV loads. Published cohorts of hospitalized patients infected with HIV-1 and suspected TB refer a prevalence between 9% and 38% in a single blood culture. A single blood culture for TB underestimates the prevalence of MTB BSI by approximately a third. Additional blood cultures within the same 24-hour period increase the diagnostic yield in a similar proportion to non-mycobacterial BSI. We recommend, financial resources permitting, to obtain at least 2 blood cultures.[8] Most ambits with high HIV-TB loads lack access to routine TB blood cultures. Evaluation of the relative cost-benefit of additional TB blood cultures would be of interest, particularly when compared with other samples, such as induced

sputum or urine Xpert® MTB / RIF Ultra, and the lipoarabinomannan urine analysis, that are more accessible in low-income settings.

3. Molecular Diagnosis

3.1 Tests supported by the WHO

3.1.1 Next-generation Xpert tests

Xpert MTB/RIF (Xpert). Xpert, a NAAT assay, allows for the rapid identification of the MTB complex (MTBC) and rifampicin resistance. Its sensitivity is, however, only limited to sputum samples. But the use of Xpert in bronchoalveolar lavage samples has a high diagnostic yield in PTB (except in children) and may serve as a rapid diagnostic tool.[9]

Xpert Ultra. The microbiologic confirmation of PTB in children is very important since clinical and radiological diagnoses lack specificity. A new Xpert MTB/RIF Ultra test (Xpert Ultra), has a superior detection threshold and sensitivity than Xpert in adults,[10] and it is more exact in induced sputum when diagnosing pediatric PTB.[11] It has low sensitivity for the diagnosis of pleural TB in adults.[12] In 2017, the WHO recommended it as the initial diagnostic test for TB in adults and children, even above smears and culture.[13]

3.1.2 Made in India: Truelab by Molbio

Truenat® MTB, Truenat® MTB Plus, and Truenat® MTB-Rif Dx (Molbio Diagnostics, Goa, India) are real-time PCR assays based on chips, for the detection of TB, and that yield results in 1 hour on the mobile Truelab® platform (Molbio Diagnostics, Goa, India). It is now used in India. Truenat is characterized as a more affordable alternative to Xpert[14], and it is manufactured in India. In December 2019, WHO convened a reunion of the Group developing the Guidelines for the recommended use of Truenat and other rapid molecular tests in trials. Subsequent communications reported that Truenat MTB, MTB Plus, and MTB-Rif Dx had comparable sensitivities and specificities to Xpert MTB / RIF and Ultra in the detection of TB and RIF resistance, although this was based on a provisional analysis of results from a multicenter study that is still underway.[15]

3.2 Emerging technologies

3.2.1 Xpert XDR (Extensively drug-resistant tuberculosis)

Another PCR-based test has been designed for use in the GeneXpert and Omni platforms, to simultaneously detect mutations associated with resistance to various TB drugs, first- and second-line, or extensively drug-resistant tuberculosis (XDR-TB). In tests of phenotype sensitivity, a prototype version of the Xpert XDR cartridge had the following sensitivities: 83.3% (95% CI 77.1-88.5) for isoniazid, 88.4% (80.2-94.1) for ofloxacin, 96,2% (87.0-99.5) for moxifloxacin at a critical concentration of 2,0 µg per milliliter, 71.4% (56.7-83.4) for kanamycin and 70.7% (54.5-83.9) for amikacin.[16]

3.2.2 GeneXpert Omni and other point-of-care (POC) devices

GeneXpert was originally designed for use at the district or sub-district level. Although efforts were made to use this technology in the lower health system échelons, it was soon obvious that microscopy centers in countries with a high TB load, frequently lacked the necessary infrastructure, including continuous control of power and temperature. The POC GeneXpert Omni platform is a long-expected development since it will allow the use of Xpert MTB / RIF and Ultra decentralized locations (i.e., primary care centers). Although its launching has been

repeatedly delayed, Omni promises to be a real POC platform, with a battery lasting two days and not requiring a tablet or a computer.[17] The first instruments will be available in 2021, and Omni will be able to execute Ultra and any other available Xpert cartridge.

Other POC NAATs are also under development. For example, Q-POC from QuantuMDx (Newcastle-upon-Tyne, United Kingdom) is a battery-operated PCR POC system that has promised to provide TB testing results in less than 30 minutes. It has been evaluated in combination with oral swabs and, in preliminary studies, its sensitivity and specificity were similar to Xpert´s.[18]

3.2.3 Indigenous Chinese diagnostics

As in the case of Molbio in India, Chinese biotechnology enterprises have used their own experience to develop TB NAAT in their own country. These companies have conformed to the China Food and Drug Administration (CFDA) norms, they have been approved and have implemented the tests at the national level. However, none of these technologies have been reviewed by the WHO, so their adoption in other countries is limited. These tests are: cross-priming amplification, Isothermal amplification of MTB 16S RNA,[19] PCR, melt curve analysis and PCR, hybridization.[20]

3.2.4 High-throughput solutions: centralized diagnostic tests

These tests have been recently developed for the diagnosis of TB and the detection of drug resistance, and they are currently under evaluation by the WHO (Abbott Molecular, Abbott Park, USA), Hain FluoroType® MTB (Hain Lifescience, Nehren, Germany), Hain FluoroType® MTDBR (Hain Lifescience, Nehren, Germany), Roche Cobas® MTB (Roche, Rotkreuz, Switzerland), and BD Max® MDR-TB (BD, Franklin Lakes, USA). These are assays conducted on platforms for several established disease entities such as the human immunodeficiency virus (HIV), human papillomavirus (HPV), and hepatitis C virus (HCV).[21]

Real-time MTB is a multiplex NAAT directed against the MTB IS6100 and PAB genes, with a limit of detection (LOD) of 17 CFU/ml. Up to 96 respiratory samples can be inactivated and processed by the Abbott m2000 platform per run, with good sensitivities and specificities in the detection of both TB and resistance.[21]

Another centralized test is the semiautomatic FluoroType MTB, a PCR assay based on a probe and run in the Hain Fluorocycler platform. Decontamination, sample preparation, and DNA isolation must be manually performed, and require 30 minutes of practical time, while the entire procedure takes 4 hours until final results are obtained; it has good sensitivity and specificity. [21]

The Cobas6800/8800 MTB assay is run on the high-performance Cobas 8800 platform and can analyze 960 samples in 8 h. A manufacturer internal study of 744 samples, reported a sensitivity and specificity of 95% (95% CI: 92-97) and 98% (95% CI: 96-99), respectively.[22]

Finally, the Max MDR-TB test runs on the BD Max platform, using the RNA target gene MTB 16S. Up to 24 samples are manually decontaminated and prepared before extraction and amplification with the Max MDR-TB assay. The time until final results are obtained is 4 hours and it has good sensitivity and specificity.[23]

Centralized TB analyses are promising due to their high diagnostic precision and their ability to run a large number of samples simultaneously; their automation decreases the risk of infection from respiratory samples

among health workers and laboratory personnel. The development of centralized assays is quite solid when used with platforms such as MeltPro (Zeesan Biotech, Xiamen, China), Seegene (Seoul, South Korea), and MolecuTech (YD Diagnostics, Seoul, South Korea), which are currently under regulatory evaluation.[24] Regardless, contamination is still possible with these assays, and quality guarantee is critical. Also, the cost of each test has not been made public.

3.2.5 Next-generation sequencing (NGS)

NGS is becoming a promising option as a TB DST and yields faster results than the traditional phenotype culture or culture-based tests. Unlike assays based on probes in which detection is limited to specific probe targets, assays based on NGS can provide detailed and precise sequence information on complete genomes, as in whole-genome sequencing (WGS), or on multiple gene regions of interest, with the targeted NGS.[25]

Recognizing the value of NGS, the WHO has published guidelines on the role of sequencing in the detection of mutations associated with TB drug resistance[25], and a consensus based on Target Product Profile on sequencing. In 2019, a TB sequencing database named ReSeqTB was established by the WHO to cure, standardize and unify DST genotype and phenotype as well as metadata on drug-resistant TB (DR-TB).[26]

Sequencing is currently implemented successfully in DR-TB surveillance in at least seven countries: Azerbaijan, Bangladesh, Belarus, Pakistan, the Philippines, South Africa, and Ukraine.[27] Some low load health systems, including the United Kingdom (British Healthcare System), the Netherlands, and the state of New York have gone from phenotype culture to WGS in DST, to first-line drugs.[28] South Africa has implemented and integrated drug resistance sequencing into its national surveillance program as an alternative to phenotype DST, and they are considering its potential future in the management of TB in the laboratory and in transmission research.[29]

3.2.6 Potential for integrating NAAT testing for TB and COVID-19

Throughout the world, health systems have been disrupted by the COVID-19 pandemic, but it is critical to avoid neglecting other diseases such as TB that are not currently the center of attention.[30] One study suggests that a non-intended result of the pandemic-related hindrances is that there will be 1.5 million TB-related deaths between 2020-2025.[31] The recently launched Xpert Xpress® SARS-CoV-2 (Cepheid, Sunnyvale, USA) could allow low- and median-income countries to increase their ability to conduct COVID-19 testing since many already have GeneXpert webs.[32] However, some are concerned because an increase in COVID-19 testing in the GeneXpert system may do so at the expense of TB tests (LMICs) that depend on Xpert MTB/RIF.[30,32]

4. Immunological Diagnosis

TB diagnosis is fundamentally focused on LTBI, so tests are meant to identify individuals at risk of developing ATB since they would benefit from timely treatment or prophylaxis.[2]

During immune response (IR) activation in LTBI, a cytokine cascade is produced, that is crucial to the development of TB. A key cytokine in infection control is interferon-γ (INF-γ), produced when MTB activates the inflammatory process in which macrophages phagocytize MTB and release cytokines that mainly attract neutrophils, macrophages, and T lymphocytes that in turn, secrete tumor necrosis

factor alfa (TNF-α), INF-γ, and interleukins such as IL-6, etc. At this point, TB infection is established.

Further, some have attempted to use cellular phenotype biomarkers to differentiate the various stages of LTBI by multiparametric flow cytometry, dividing ATB patients, non-infected (NoTBI) and LTBI; they reported that patients with active TB (ATB) have a higher Monocyte-Lymphocyte and Neutrophil-Lymphocyte ratio than patients with LTBI and NoTBI.[33] They also identified different cell subpopulations that could potentially help establish the diagnosis of TB, such as CD4 T_{CM} cells and their expression of CD154 after activation by specific TB antigens.

Another study evaluated the household contacts of TB patients. These individuals had negative IGRA and tuberculin skin test (TST), "resisting" the development of LTBI. They showed that "resisters" had IgM, class-switched IgG antibody responses, and non-IFN-γ T cell responses to the specific proteins -MTB, ESAT6, and CFP10, evidence of immune exposure to MTB. Their antibodies also displayed increased avidity and different IgG Fc profiles, specific to MTB. They demonstrated that these "resistant" individuals developed non-canonical humoral and cellular immunity to MTB. They also developed specific B and T cell immunity to MTB with CD4 Th responses linked to quantitative and qualitative differences in the IgG profile against ESAT6 / CFP10. These individuals did not harbor a defect in IFN-γ production, they developed a selective T cell response that did not produce IFN-γ against MTB, and it is possible that they sensitized mycobacterial antigens through BCG or environmental mycobacteria before MTB exposure.[34]

Further, analysis of multiple cytokines was conducted on culture supernatants after 6 days and they were restimulated *in vitro* with MTB IGRA and latency-associated antigens (Rv2628, Rv1733), in TB patients and their asymptomatic contacts. IL-6 had the greatest sensitivity to improve the detection of MTB infection and classify TB patients and healthy contacts.[35]

The predictive value of 13 analytes selected as potential biomarkers was investigated; they included cytokines and chemokines measured with QuantiFERON Gold in tube (QFT) in plasma samples from patients coinfected with TB and HIV. They used MilliplexTM kits (Millipore, St Charles, MO) on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA). They included patients with active ATB, patients that developed TB during a 4-year follow-up, and controls. The greatest differences were observed in the C-X-C residue of chemokine 10 (CXCL10), interleukin-2 (IL-2), IL-1α, and transforming growth factor-α (TGF-α). They suggested that biomarker combinations were required to detect ATB in coinfected individuals.[36]

To date, the identification of LTBI is a major challenge, but immune mediators are most helpful in establishing the diagnosis although they should be cautiously interpreted since the immune response to MTB varies in different individuals.

5. Conclusion

Advances in the molecular diagnosis of TB over the past decade, have led to very precise and rapid TB tests, superior to microbiological testing; emerging technologies appear to follow this tendency. In some aspects, NAAT have had a positive clinical impact. As long as care in high load TB settings remains weak or fragmented, diagnostic tests alone cannot decrease mortality or disease recurrence, and the evaluations of the clinical relevance of NAAT will continue to yield no results. Issues such as NAAT sub par use, empirical treatment

of individuals suspected of harboring TB, and the number of patients lost to follow-up, decrease the potential beneficial effect of diagnostic tests. A timely diagnosis is pivotal, so all risk factors for the development of TB must be well established, and appropriate diagnostic testing must be conducted as soon as possible. Also, the identification and treatment of LTBI may temper the risk of disease and help prepare potential strategies to control TB in the community.

References

1. World Health Organization. 2020. Global tuberculosis report 2020. Geneva: World Health Organization, WHO Press, Geneva

2. García-Elorriaga G, del Rey-Pineda G. Practical and laboratory diagnosis of tuberculosis: From sputum smear to molecular biology. Switzerland: Springer, 2015. ISBN 978-3-319-20478-9

3. García-Elorriaga G, del Rey-Pineda G. Current Research in the Laboratory Diagnosis of Tuberculosis. SMGroup, 2016. ISBN: 978-1-944685-00-3

4. Fennelly KP, Acuna-Villaorduna C, Jones-Lopez E, Lindsley WG, Milton DK. Microbial Aerosols: New Diagnostic Specimens for Pulmonary Infections.Chest. 2020; 157:540-546. doi: 10.1016/j.chest.2019.10.012

5. Costa RRD, Silva SFD, Fochat RC, Macedo RL, Pereira TV, Silva MR et al. Comparison between Ogawa-Kudoh and modified Petroff techniques for mycobacteria cultivation in the diagnosis of pulmonary tuberculosis. Einstein (Sao Paulo). 2018; 16:eAO4214. doi: 10.1590/S1679-45082018AO4214

6. Asmar S, Drancourt M. Rapid culture-based diagnosis of pulmonary tuberculosis in developed and developing countries. Front Microbiol. 2015; 6:1184. doi: 10.3389/fmicb.2015.01184

7. Alva A, Aquino F, Gilman RH, Olivares C, Requena D, Gutiérrez AH et al. Morphological Characterization of Mycobacterium tuberculosis in a MODS Culture for an Automatic Diagnostics through Pattern Recognition. PLoS One. 2013; 8::e82809. doi: 10.1371/journal.pone.0082809

8. Barr DA, Kerkhoff AD, Schutz C, Ward AM, Davies GR, Wilkinson RJ et al. HIV-associated *M. tuberculosis* blood stream infection is under-diagnosed by a single blood culture. J Clin Microbiol. 2018; 56:e01914-17. doi: 10.1128/JCM.01914-17

9. Liu HC, Gao YL, Li DF, Zao XY, Pan YQ, Chang-Tai Zhu CT. Value of Xpert MTB/RIF using bronchoalveolar lavage fluid for the diagnosis of pulmonary tuberculosis: A systematic review and meta-analysis. J Clin Microbiol. 2020; pii: JCM.02170-20. doi: 10.1128/JCM.02170

10. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J et al. The New Xpert MTB/RIF Ultra: improving detection of mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. MBio. 2017; 8:e00812–e00817

11. Nicol MP, Workman L, Prins M, Bateman L, Ghebrekristos Y, Mbhele S et al. Accuracy of Xpert MTB/RIF Ultra for the diagnosis of pulmonary tuberculosis in children. Pediatr Infect Dis J. 2018 ; 37:e261-e263. doi: 10.1097/INF.0000000000001960

12. Meldau R, Randall P, Pooran A, Limberis J, Makambwa E, Dhansay M et al. Same day tools, including Xpert Ultra and unstimulated IFN-1 γ, for the rapid diagnosis of pleural tuberculosis – a prospective observational study. J Clin Microbiol. 2019; 26; 57:e00614-19. doi: 10.1128/JCM.00614-19

13. World Health Organization. 2017. Next-generation Xpert® MTB/RIF Ultra assay recommended by WHO. World Health Organization, Geneva.

14. Pai M. 2020. Global health technologies: time to re-think the 'trickle down' model. Forbes.

15. World Health Organization. 2020. Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication. Policy update. World Health Organization, Geneva

16. 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; 377:1043-1054

17. Treatment Action Group. 2019. 2019 Pipeline Report: Tuberculosis Diagnostics.Treatment Action Group, Group TA, New York.

18. Ortega C, Wood R, Murton H, Andama A, Cattamanchi A, Dixon R et al. Diagnosis of pulmonary tuberculosis by oral swab analysis (OSA): optimisation and development of non-sputum, point-of-care methods. Int J Tuberc Lung Dis. 2019; 23:S211

19. Deng S, Sun Y, Xia H, Liu Z, Gao L, Yang J et al. Accuracy of Commercial Molecular Diagnostics for the Detection of Pulmonary Tuberculosis in China: A Systematic Review. Sci Rep. 2019; 9:4553

20. Sun Y, Gao L, Xia H, Yang Z, Deng S, Yang J. Accuracy of molecular diagnostic tests for drug-resistant tuberculosis detection in China: a systematic review. Int J Tuberc Lung Dis. 2019; 23:931-942

21. Kohli M, MacLean E, Schumacher SG, Pai M, Denkinger CM. Under review. Diagnostic accuracy of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid: A systematic review and meta-analysis. Eur Respir J. 2020; doi: 10.1183/13993003.00747-2020

22. Roche Molecular Systems I. 2018. Cobas(R) MTB: Nucleic acid test for use on the Cobas(R) 6800/8800 systems. Roche Molecular Systems, Basel

23. Rocchetti TT, Silbert S, Gostnell A, Kubasek C, Widen R. Validation of a Multiplex Real-Time PCR Assay for Detection of Mycobacterium spp., Mycobacterium tuberculosis Complex, and Mycobacterium avium Complex Directly from Clinical Samples by Use of the BD Max Open System. J Clin Microbiol. 2016; 54:1644-7

24. FIND. 2020. Diagnostics Pipeline Tracker: Tuberculosis, *on* FIND. https://www.finddx.org/dx-pipeline-status/. Accessed

25. World Health Organization. 2018. Technical guide on next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex. World Health Organization, Organization WH, Geneva

26. Organization WH. 2019. Relational sequencing TB data platform [website]. World Health Organization, Geneva.

27. Zignol M, Cabibbe AM, Dean AS, Glaziou P, Alikhanova N, Ama Cet al. Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. Lancet Infect Dis. 2018; 18:675-683.

28. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M et al. Whole genome sequencing of Mycobacterium tuberculosis: current standards and open issues. Nat Rev Microbiol. 2019; 17:533-545

29. National Institute for Communicable Diseases. 2018. National Institute for Communicable Diseases Annual Overview 2018/2019. National Institute for Communicable Diseases, Capetown.

30. Venkatesan P. COVID-19 diagnostics- not at the expense of other diseases. Lancet Microbe. 2020; 1:e64 doi: 10.1016/S2666-5247(20)30041-0

31. Stop TB Partnership. 2020. The potential impact of the COVID-19 response on tuberculosis in high-burden countries: a modelling analysis. Stop TB Partnership, Geneva

32. United States Food and Drug Administration. 2020. Coronavirus (COVID-19) Update: FDA Issues first Emergency Use Authorization for Point of Care Diagnostic. United States Food and Drug Administration, Atlanta

33. Estévez O, Anibarro L, Garet E, Martínez A, Pena A, Barcia L et al. Multi-parameter Flow cytometry immunophenotyping distinguishes different stages of tuberculosis infection. J Infect. 2020; 81:57-71. DOI: 10.1016/j.jinf.2020.03.064

34. Lu LL, Smith MT, Yu KKQ, Luedemann C, Suscovich TJ, Grace PS et al. IFN-γ independent immune markers of *Mycobacterium tuberculosis* exposure. Nat Med. 2019, 25:977-987. doi: 10.1038/s41591-019-0441-3

35. Adankwah E, Nausch N, Minadzi D, Abass MK, Franken KLMC, Ottenhoff THM et al. Interleukin-6 and Mycobacterium tuberculosis dormancy antigens improve diagnosis of tuberculosis. J Infect. 2020; 0163-4453(20)30729-5. doi: 10.1016/j.jinf.2020.11.032.

36. Lesosky M, Rangaka MX, Pienaar C, Coussens AK, Goliath R, Mathee S et al. Plasma biomarkers to detect prevalent or predict progressive tuberculosis associated with Human Immunodeficiency Virus–1. Clin Infect Dis. 2019; 69:295-305. doi: 10.1093/cid/ciy823

