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Update on the diagnosis of tuberculosis

Irina Kontsevaya [1,2,3,4,*], Andrea Maurizio Cabibbe [5], Daniela Maria Cirillo [5], Andrew R. DiNardo [6,7], Nicole Frahm [8], Stephen H. Gillespie [9], David Holtzman [8, 10], Lennard Meiwes [1,2,3], Elisa Petruccioli [11], Maja Reimann [1,2,3], Morten Ruhwald [12], Wilber Sabiiti [9], Francesca Saluzzo [5,13], Elisa Tagliani [5], Delia Goletti [11]

[1)] Division of Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany

[2)] German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany

[3)] Respiratory Medicine & International Health, University of Lübeck, Lübeck, Germany

[4)] Department of Infectious Disease, Faculty of Medicine, Imperial College London, London, United Kingdom

[5)] Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

[6)] Global TB Program, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas, USA

[7)] Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, the Netherlands

[8)] Bill & Melinda Gates Medical Research Institute, Cambridge, Massachusetts, USA

[9)] School of Medicine, University of St Andrews, St Andrews, United Kingdom

[10)] Section of Infectious Diseases, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[11)] Translational Research Unit, National Institute for Infectious Diseases (INMI) "Lazzaro Spallanzani" - IRCCS, Rome, Italy

[12)] FIND, Geneva, Switzerland

[13)] Vita-Salute San Raffaele University, Milan, Italy

Corresponding author: Irina Kontsevaya. Address: Research Center Borstel, 35 Parkallee, 23845, Borstel, Germany. Email: ikontsevaya@fz-borstel.de. Tel: +49 (4537) 188 3677

Abstract

Background: Tuberculosis remains a global public health threat, and the development of rapid and precise diagnostic tools is the key to enabling the early start of treatment, monitoring response to treatment, and preventing the spread of the disease.

Objective: An overview of recent progress in host- and pathogen-based tuberculosis diagnostics.

Sources: We conducted a PubMed search of recent relevant articles and guidelines on tuberculosis screening and diagnosis.

Content: An overview of currently used methods and perspectives in the following areas of tuberculosis diagnostics is provided: immune-based diagnostics, X-ray, clinical symptoms and scores, cough detection, culture of *Mycobacterium tuberculosis* and identifying its resistance profile using phenotypic and genotypic methods, including next generation sequencing, sputum- and non-sputum-based molecular diagnosis of tuberculosis and monitoring of response to treatment.

Implications: A brief overview of the most relevant advances and changes in international guidelines regarding screening and diagnosing tuberculosis is provided in this review. It aims at reviewing all relevant areas of diagnostics, including both pathogen- and host-based methods.

Introduction

Tuberculosis (TB) remains a global public health threat that requires rapid and precise diagnostic tools to enable the early start of treatment and prevent the spread of the disease. National TB programmes were affected by the COVID-19 pandemic with a large drop in the number of people newly diagnosed with TB [1]. However, the pandemic has also stimulated rapid growth in the field of diagnostics for infectious diseases, with many novel tests and platforms aiming at rapid and precise detection of the pathogen, which has also boosted TB diagnostics. Overall, significant progress has been made in the past decades in diagnosing stages of TB from TB infection to TB disease. This review gives an overview of recent progress in host- and pathogen-based TB diagnostics. For that, we conducted a PubMed search of relevant articles focusing on articles published in the last decade as well as the most recent updates of guidelines on TB screening and diagnosis.

Diagnostics of tuberculosis infection

Immune-based diagnostics of tuberculosis infection

TB infection (TBI) is a state in which we detect an immune response to *Mycobacterium tuberculosis* (Mtb) in the absence of clinical, microbiological and radiological signs of disease (Figure 1). TBI can progress to TB disease via stages of incipient TB, when there are still no microbiological, radiological, or clinical signs of disease but a Mtb-specific immune response is detected and the TB progression test can be positive, and subclinical TB when radiological and/or microbiological signs of TB are detected but there are still no clinical symptoms specific for TB. With the progression to TB disease, clinical symptoms appear.

In the state of TBI, Mtb is suspected to be in a low-replicative stage and in the absence of standard technologies to detect it, we measure the Mtb-specific immune response as an indirect assessment of infection, using tuberculin skin test (TST) and interferon (IFN)-γ release assays (IGRAs) [2]. TST involves intradermal injection of purified protein derivative (PPD) causing a delayed type

immune reaction determining an induration; assay score is based on the size of immune infiltrate after 48-72 hours. TST has a low cost, does not require a laboratory setting and is useful in large screening. However, the specificity for TBI diagnosis is affected by the PPD cross-reaction with non-tuberculous and tuberculous Mycobacteria, including Bacillus Calmette et Guerin [3]. Specificity is improved using Mtb-specific antigens (ESAT-6, CFP-10), as in new skin tests [Cy-Tb (Serum Institute of India, India), Diaskintest (Generium, Russia), and EC skin test (Anhui Zhifei Longcom, China)] [4, 5].

IGRAs are based on IFN-γ detection in response to Mtb-specific antigens (ESAT-6, CFP-10). QuantiFERON-TB Gold Plus (Qiagen, Germany) based on whole blood and ELISA and T-SPOT TB (Oxford Immunotec, UK) based on isolated lymphocytes/monocytes and ELISpot are worldwide used IGRAs that require an equipped laboratory and trained staff [3, 6].

The WHO is currently evaluating multiple next generation IGRAs as "next in class". They are based on different methodologies such as chemiluminescence, automated enzyme-linked immunofluorescent assay, lateral flow technique, or non-IGRA testing (Table 1).

Although IGRA and TST are widespread and recommended for TBI diagnosis [2], they do not distinguish infection from disease [3, 6] and poorly predict TB progression [7]. An increase of thresholds for QFT-GIT, T-SPOT.TB, and TST may increase the positive predictive value for incident TB at the cost of sensitivity reduction [7] without improving accuracy for routine application. Regarding the new skin tests and IGRAs, we do not expect a higher accuracy compared to routine IGRAs because based on the same Mtb-specific antigens [2]. Alternative experimental IGRAs involve antigens different from ESAT-6 and CFP-10, such as heparin-binding hemagglutinin antigen associated with Mtb containment, as reported in children, adults, people living with HIV (PLHIV) [8-10]. Other approaches are based on antibody detection [11].

Table 1

Tools for the diagnosis of TBI in the past and present

	Description	Skin tests	IGRAs	
Present/ Past	**Commercial test**	***TST***	***QuantiFERON-TB Gold Plus*** *(Qiagen)*	***T-SPOT TB*** *(Oxford Immunotec)*
	Characteristics	• PPD based	• ELISA • ESAT-6/CFP10 based • Whole blood based	• ELISPOT • ESAT-6/CFP10 based • PBMC based
	Main benefits	• No laboratory needed	• High specificity	• High specificity
	Main limitations	• Low specificity • Poor sensitivity in immune-compromised individuals	• Equipped laboratory needed • Poor sensitivity in immune-compromised individuals	• Equipped laboratory needed • Poor sensitivity in immune-compromised individuals
	WHO endorsement	• WHO endorsed [12]	• WHO endorsed: ***Qiagen QuantiFERON-TB Gold Plus*** performance is comparable to that of WHO-recommended IGRAs for the detection of TB infection [13]	• WHO endorsed [12]
Present	**Commercial test**	***- Diaskintest*** *(Generium)* ***- EC skin test*** *(Anhui Zhifei Longcom)* ***- Cy-Tb*** *(Serum Institute of India)*	***- Liaison QuantiFERON Plus:*** *chemiluminescence (Qiagen)* ***- AdvanSure TB-IGRA:*** *chemiluminescence (LG Chem)* ***- WANTAI TB-IGRA*** *ELISA, three tubes based (Beijing Wantai)* ***- T-SPOT.TB 8 with T-Cell Select (T-Cell Select)*** *simplified procedure to automatically isolate mononuclear cells from whole blood (Oxford Immunotec)*	***- QIAreach* QuantiFERON-TB*** *(Qiagen)* ***- ichroma IGRA-TB*** *(Boditech)* ***- STANDARD F TB-Feron FIA*** *(SD Biosensor)*
	Characteristics	• ESAT-6/CFP-10 based	• Alternative methodology to run large volume of sample or automated workstation • ESAT-6/CFP10 based • Whole blood based	• Lateral flow test • ESAT-6/CFP10 based • Whole blood based
	Main benefits	• High specificity	• High specificity	• High specificity • No laboratory needed

	• No laboratory needed		
Main limitations	• Poor sensitivity in immune-compromised individuals	• Equipped laboratory needed • Poor sensitivity in immune-compromised individuals	• Poor sensitivity in immune-compromised individuals
WHO endorsement	• WHO endorsed; Recommendation: Mtb antigen-based skin tests (TBSTs) may be used to test for TB infection. Conditional recommendation for the intervention, very low certainty of the evidence [12]	• ***Liaison QuantiFERON Plus, AdvanSure TB-IGRA:*** WHO evaluation not available [12] • ***WANTAI TB-IGRA:*** WHO endorsed, the performance is comparable to that of WHO-recommended IGRAs for the detection of TB infection [13] • ***T-SPOT.TB 8 with T-Cell Select (T-Cell Select)*** not WHO endorsed: based on available data, could not be adequately compared with WHO-recommended IGRAs for detection of TB infection [13].	• ***STANDARD F TB-Feron FIA:*** not WHO endorsed; based on available data, it could not be adequately compared with WHO-recommended IGRAs for detection of TB infection [13]. • ***ichroma IGRA-TB****:* WHO evaluation not available [12]

ELISA, enzyme linked immunosorbent assay; ELISPOT, Enzyme-linked ImmunoSPOT; PPD, protein purified derivative; TBI, tuberculosis infection; TST, tuberculin skin test; *not available yet.

Diagnostics of tuberculosis disease

Clinical symptoms and scores, chest X-ray, and cough detection

The World Health Organization (WHO) 4-symptom TB screen is recommended for active case finding in PLHIV of all ages, close contacts of TB cases, and other targeted populations separately or in combination with chest X-ray (CXR), molecular WHO-recommended rapid diagnostic tests (mWRDs) for TB, and/or immune response markers such as C-reactive protein [12]. The sensitivity and specificity of the 4-symptom screen varies significantly depending on antiretroviral status and CD4 count in

PLHIV, age, and population TB burden, among other factors [14]. Multiple clinical scores have been designed for adults to improve upon the performance characteristics of the WHO 4-symptom screen or better inform the post-test probability of a confirmed TB diagnosis in an individual screening positive on the WHO 4-symptom screen through the addition of other clinical symptoms or signs or anthropometric measurements [15]. These scores can help prioritise use of constrained testing resources or guide clinical management before test results are available [16-22] though they require external validation before broader use [15]. Several paediatric scores incorporating clinical signs and symptoms, exposure history, CXR findings, TST results, and/or lab results have been developed to aid clinicians with diagnosis due to the difficulty of bacteriological confirmation of TB disease in children [23, 24].

CXR is an important TB diagnostic tool in individuals with and without TB symptoms. Several TB-specific computer-assisted detection (CAD) software applications using artificial intelligence have been demonstrated to improve the sensitivity and specificity of CXR in both use cases and are now recommended by the WHO [12, 25]. Portable ultralight CXR machines combined with CAD interpretation have the potential to make CXR more accessible for populations in greatest need of improved TB diagnostics. Current CAD software applications are not recommended for use in TB diagnosis in children <15 years because CXRs from this sub-population were not used in their development and TB often causes different CXR findings in children [12].

Cough is often a hallmark symptom of pulmonary TB and assessing cough and its decline following initiation of treatment is crucial for clinical care. Novel technologies allow for accurate counting and characterization of cough [26]. Numerous companies are taking advantage of cell phone microphones to collect cough sounds by applying AI-driven algorithms for their identification and enumeration (https://www.hyfe.ai/; https://www.resapphealth.com.au/technology/; https://www.nuvoair.com/). Further advancement of these technologies may provide enough differentiation of cough sounds to contribute to the accurate diagnosis of TB and other pulmonary diseases though the absence of cough in a notable minority of individuals with bacteriologically confirmed TB will likely limit the scope of their impact on TB diagnosis [27].

Sputum-based diagnostics of tuberculosis

Sputum has long been the most used sample in TB diagnosis. Traditionally, the diagnostic aim has been to identify the presence or absence of disease, the susceptibility pattern of the organism, and to measure the response to treatment.

Mycobacterium tuberculosis culture

Liquid automated culture performed through BACTEC MGIT (Becton Dickinson, USA) remains deeply embedded in the TB diagnostic algorithm, being the most sensitive confirmatory method available, especially in the case of extrapulmonary TB. According to current recommendations, culture should be performed whenever feasible on all first diagnostic samples and for monthly treatment monitoring [28].

Molecular diagnostics of tuberculosis

Xpert (Cepheid, USA) provides a real-time polymerase chain reaction to detect the presence of Mtb as well as rifampicin resistance in a single automated cartridge [29]. This integration provides both direct diagnostic information as well as a guide to empirical therapy that is easy to deploy. Supplemented by a second Xpert MDR/XDR test that detects resistance to isoniazid, fluoroquinolones, amikacin, kanamycin, capreomycin and ethionamide it may provide a comprehensive guide to therapy in resistance cases [30].

Drug susceptibility testing of *Mycobacterium tuberculosis*

Phenotypic drug susceptibility testing

Mtb strains obtained through culture can be further characterised through phenotypic drug susceptibility testing (pDST), minimal inhibitory concentration (MIC) determination and next generation sequencing. pDST is usually performed in MGIT™ using defined critical concentrations

(CCs), as a clinical breakpoint has currently only been established for moxifloxacin [31]. Non-commercial pDST assays include microscopic observation of drug susceptibility (MODS), thin-layer agar (TLA), or colorimetric redox indicator (CRI), among others [32].

pDST presents several constraints and the advent of reliable, accurate and rapid molecular methods for the detection of rifampicin and isoniazid resistance has led to a decline in the use of pDST for these TB cornerstone drugs [33].

Among first line drugs, pyrazinamide pDST also shows several technical hurdles and is hampered by a different MIC distribution of Lineage 1 strains [34].

Regarding new and repurposed drugs, pDST for bedaquiline and linezolid at WHO-recommended CC should be performed when resistance is suspected and for surveillance at population level [35]. For pretomanid a MIC bimodal distribution has been observed associated with Lineage 1 strains and a consensus on CC for this drug has yet to be reached [36].

A standardisation of pDST in MGIT against the EUCAST Broth MicroDilution (BMD) in microtiter plates protocol is ongoing as MIC determination could represent a more effective strategy (Table 2) to monitor resistance trends [37]. A suitable plate layout was proposed by the WHO; plates are not yet available, but a validation round is planned by 2024.

Table 2

Advantages and disadvantages of the use of MGIT or EUCAST Broth MicroDilution in microtiter plates to perform phenotypic drug susceptibility testing

	Advantages	**Disadvantages**
MGIT	Standardised method, automated reading and reporting	Cost, needs to be set up in one tube at a time, results are available by CC only, difficult to interpret for new drugs
BMD in microtiter plates	Provide MIC, possibility to monitor resistance trends	Mostly manual, amount of inoculum may influence results, different reading time

	especially for new drugs, set up of several drugs at same time, cost

BMD, Broth MicroDilution; CC, critical concentration; MGIT, Mycobacteria Growth Indicator Tube; MIC, minimal inhibitory concentration.

Genotypic drug susceptibility testing

In 2021, following the systematic review of diagnostics accuracy, the WHO recommended the use of three classes of nucleic acid amplification tests (NAATs), expanding the range of rapid diagnostics that allow for rapid detection of tuberculosis and resistance of bacteria to antituberculosis drugs [33]. However, none of currently recommended genotypic DST assays determine resistance to new and repurposed drugs (Table 3). A number of molecular tests are available on market but not evaluated by the WHO yet, for example, AccuPower TB&MDR and XDR-TB (Bioneer, Korea), Genechip MDR test (Capital Bio, China), or mfloDx MDR-TB (EMPE Diagnostics, Sweden).

Table 3

Classes of technologies and associated products currently recommended by the WHO for rapid diagnosis of tuberculosis and resistance to antituberculous drugs (modified from [33])

Technology class	Products included in the WHO evaluation	Strengths	Limitations
	Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid)	• Point-of-care test • Rapid and easy to perform • Detects Mtb and rifampicin resistance • Requires minimal laboratory infrastructure	Sensitivity is suboptimal in specific groups, e.g. smear-negative or PLHIV

	Truenat™ MTB, MTB Plus and MTB-RIF Dx (Molbio)	• Rapid and easy to perform • Detects Mtb and rifampicin resistance • Can be performed in peripheral laboratories • Requires minimal laboratory infrastructure and training of staff • Battery-operated device	• More complex test from the user perspective • Limited data on diagnostic accuracy in specific groups, e.g. PLHIV, extrapulmonary TB
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)	• High throughput • Largely automated • Detect Mtb and resistance to rifampicin and isoniazid	• May require an initial manual specimen treatment step • require medical laboratories with biosafety measures in place and test-specific equipment • Require well-trained, skilled and qualified laboratory staff • Require complex maintenance of equipment • Limited data on diagnostic accuracy in specific groups, e.g. PLHIV, extrapulmonary TB
	TB-LAMP (Eiken)	• Manual assay • Rapid and easy to perform • Requires little infrastructure and biosafety level	• Does not detect resistance to drugs • Relatively low sensitivity • Limited data on diagnostic accuracy in different epidemiological and geographical settings and patient populations
Antigen detection in a lateral flow format (biomarker-based detection)	Alere Determine™ TB LAM Ag (Alere)	• Non-sputum based, non-invasive, easy-to-obtain sample • Improved sensitivity in PLHIV with low CD4 count	• Does not detect resistance to drugs • Low sensitivity in HIV-negative patients • Lower sensitivity compared to second and third generation LAM test
Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)	• Point-of-care test • Rapid and easy to perform • Detects Mtb and resistance to isoniazid, fluoroquinolones, ethionamide and second-line injectable drugs (amikacin,	• Limit of detection is higher than Xpert® MTB/RIF Ultra • Not recommended for testing on samples with "Mtb complex trace detected" • Test for pre-XDR TB rather than XDR-TB

		kanamycin and capreomycin) • Requires minimal laboratory infrastructure	
Line probe assays (LPAs)	GenoType® MTBDRplus v1 and v2; GenoType® MTBDRsl, (Hain Lifescience/Bruker) Genoscholar™ NTM+MDRTB II; Genoscholar™ PZA-TB II (Nipro)	• Can be partly automated • Detect Mtb and resistance rifampicin, isoniazid, pyrazinamide, fluoroquinolones, and second-line injectable drugs (amikacin, kanamycin and capreomycin) • Perform both on sputum specimens and cultured isolates	• More complex tests from the user perspective • Limited evaluation data on non-sputum respiratory samples • Cannot determine resistance to individual drugs in the class of fluoroquinolones • Mutations that may be important in some regions are not included

Next generation sequencing

High throughput or next generation sequencing (NGS) technology raises exciting opportunities for studying the Mtb genome and for the development of future TB diagnostics [38].

The development of benchtop and even portable sequencing platforms combined with significant reduction of sequencing costs, time and workflow complexity has enabled the progressive utilisation of Mtb NGS in clinical practice and for public health [39].

As a public health tool, whole genome sequencing (WGS), i.e., sequencing of the entire bacterial genome, has been shown to provide the highest level of granularity for the detection of transmission outbreaks [40] and to monitor trends of drug resistance [41].

In 2021, the WHO published the first standardised catalogue of mutations in the Mtb complex genome and associated drug resistance using globally representative WGS data to guide end users in the interpretation of sequencing data [42]. This dataset is also a key resource for developers to support the selection of relevant targets and associated mutations to be included in sequencing-based DST. In this context, culture-free solutions based on targeted NGS (tNGS), such as the commercially available Deeplex Myc-TB (GenoScreen, France), provide comprehensive drug resistance profiles starting directly from clinical specimens and have the advantage of significantly reducing the DST turnaround

219 times, allow for the detection of minor frequency variants and subpopulations, and are less data intense
220 than WGS [43, 44]. Furthermore, other tNGS assays at late-stage development (e.g. ABL; Oxford
221 Nanopore Technologies, ONT; Clemedi) and currently being evaluated [45].

222 Another breakthrough came with the development of the third-generation sequencing
223 technologies able to generate long reads (LRS, 1-100+ kb, e.g. ONT; PacBio), as opposed to the
224 conventional short-reads (e.g., Illumina; MGI Tech; ThermoFisher Scientific) (SRS, 75–300 bp), which
225 helped to resolve hard-to-sequence regions of the Mtb genome such as large structural variations and
226 repetitive regions [46]. Even if LRS has reported higher error rate than SRS, this limitation can be
227 overcome by adopting hybrid approaches for high-quality genome assemblies [47].

228 As several options for wet and dry TB-related NGS processes are becoming available, we
229 highlight the key research needs to close current gaps for their optimal use in patient care and
230 surveillance (Table 4).

231

232 **Table 4**

233 Gaps and future directions in NGS for tuberculosis diagnosis and performing genotypic drug
234 susceptibility testing

Gaps / Future directions in TB NGS
Development of rapid, automated NGS (tNGS or WGS) workflows suitable for decentralised testing
NGS implementation in high TB burden, low-resource settings
Validation of tNGS solution on a wider array of specimen types
Development of culture-free WGS approaches overcoming limitations of tNGS
Standardisation of NGS reports for clinical decision making and link to electronic health records
Standardisation and automation of post-sequencing processes
Update of mutation catalogues, including new and repurposed drugs
Worldwide accessibility to NGS (supply)

235

NGS, next generation sequencing; tNGS, targeted next generation sequencing; WGS, whole genome sequencing.

Sputum-based assays for monitoring of response to antituberculous treatment

The tuberculosis molecular bacterial load assay (TB-MBLA) takes a different approach, targeting 16S ribosomal RNA [48]. This has a short half-life after Mtb cell death, is present in multiple copies and is thus a sensitive marker of viable count. It has been shown to be reproducible in a high-burden setting [49], and able to detect differences between treatment regimens [50].

Mtb cell wall includes lipoarabinomannan (LAM), and detection of this antigen has been used to detect the presence of organisms in sputum. Initial indications suggest that sputum LAM can be used to estimate the bacterial count at the early stages of treatment [51]. Further studies are required to show its applicability over the duration of TB therapy.

Among emerging tests, sputum incubation for 60 mins at 46^{o}C triggers the release of MPT64, an Mtb-specific protein, from live bacteria. Early small-scale studies show that the signal falls in response to treatment suggesting its diagnostic and therapeutic monitoring potential [52].

On the host side, biomarker candidates with the potential to improve treatment monitoring and determination of treatment success include transcriptomic profiling, host adaptive responses, clinical score, signs, lung function and imaging [53].

Non-sputum-based methods of tuberculosis diagnostics

Sputum remains an access barrier for TB testing in particular at the primary health care level where most patients are seeking care and replacing sputum with a simpler sample is expected to increase diagnostic yield and microbiological confirmation of TB. Tongue swabs is a leading contender as a field friendly sputum replacement test, and when combined with a sensitive molecular backend such as the

Xpert Ultra, this sample type can deliver sensitivity slightly below a sputum based test but with a simpler to obtain sample, modelled to increase number of patients detected [54].

Bioaerosol sampling capturing Mtb in exhaled breath using face masks or blow tube filters is still experimental but preliminary data suggests this sample type also has potential as a sample type to replace sputum [55, 56]. Both tongue swabs and bioaerosol sampling, as well as detection of Mtb in saliva [57], are still on early stages of development and require extensive further work.

A simple blood-based diagnostic for TB is pursued using host and bacterially derived markers. Host measurement of gene expression signatures in a finger prick sample has demonstrated high sensitivity but may prove suboptimal specific in particular outside of high endemic settings [58]. Capturing cell free DNA fragments provides direct measure of Mtb infection and has recently been shown surprisingly sensitive when coupled with a specific clustered regularly interspaced short palindromic repeats (CRISPR) based amplification and detection step in both children and adults [59].

Stool remains an attractive alternative sample type in particular for young children who have difficulty producing high quality sputum samples. A systematic review underlying the recent WHO policy recommendation of stool as an alternative sample for paediatric TB detection in the Xpert MTB/RIF and MTB/RIF Ultra system suggested acceptable usability and similar diagnostic accuracy compared with sputum-based sampling [60]. The pulmonary mucociliary escalator drains lung debris into the gastrointestinal (GI) tract and therefore both GI sampling (gastric lavage, string test, stool, rectal swab) may allow Mtb bacilli detection. Stool studies have identified both Mtb DNA and RNA (representative of viable bacilli), therefore allowing stool-based diagnostics and treatment monitoring of viable organisms [61, 62]. It remains unclear how GI and stool-based tests should augment conventional sputum-based testing.

In PLHIV, Xpert in urine increased diagnostic yield of TB [63]. Also, WHO recommends the use of urine LAM test for TB diagnosis in people with advanced HIV co-infection and low CD4 cell counts [64]. More sensitive LAM tests can also improve TB diagnosis in HIV-negative children [65]. As urine LAM can provide rapid, point-of-care diagnosis of TB it can be particularly helpful in settings

with limited resources where traditional TB diagnostic methods may not be readily available. However, the sensitivity of urine LAM for detecting TB is relatively low compared with other diagnostic tests.

Conclusions

In the past decades, TB diagnostics have made significant progress, moving from culture-based methods to more rapid and precise assays that are less labour- and time-consuming and do not require extensive high biosafety level laboratory. Moreover, the field is moving away from sputum-based assays towards less invasive, more precise methods that include biological samples easier to collect. However, for many novel assays, sufficient clinical evidence to support their use in TB diagnostics is still lacking. Large clinical studies to validate the use of novel TB diagnostic assays are urgently needed.

Transparency declaration

Conflict of interest

DG declares consulting fees from PBD Biotech, Quidel, and Eli Lilly, and personal fees from Amgen, Biomerieux, Almirall, Diasorin, Eli Lilly, Janssen, PDB Biotech, Qiagen, and Quidel. LM declares an MD stipend from German Center for Infectious Research (DZIF). All other authors declare no conflict of interest.

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Author contribution

IK designed the structure of this review. All authors wrote the manuscript, critically revised it for important intellectual content, gave final approval of the version to be published, and agree to be accountable for all aspects of this work.

Access to data

Not applicable

Figure legend

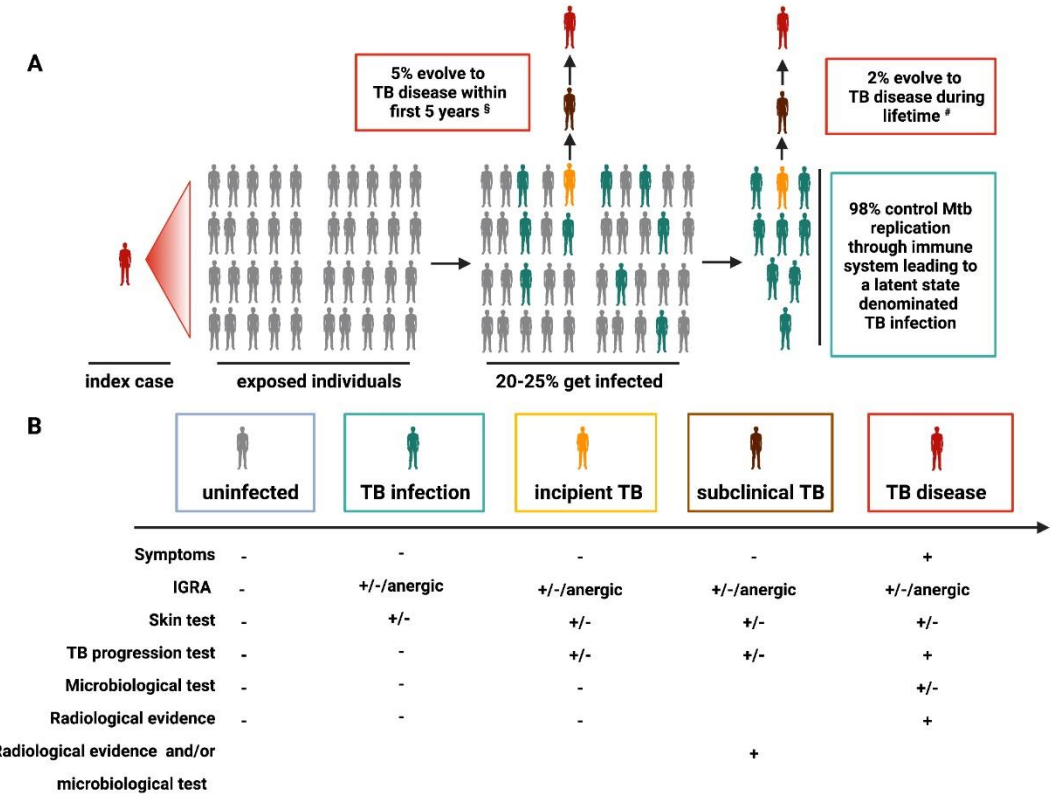

Figure 1

A) Natural history of tuberculosis and B) diagnostic tools for detection of tuberculosis infection and disease. *Mycobacterium tuberculosis* infection is characterised by different conditions strictly connected to each other: in TB infection there are no signs or symptoms of disease and in the case of immune suppression IGRAs and skin test could give a negative or anergic response (anergy is diagnosed only by IGRA); in case of incipient TB signs or symptoms of disease are absent but the bacteria are alive and replicating; individuals with subclinical TB do not have symptoms but may have radiological

or/and microbiological evidence of TB disease; patients with TB disease have classical signs and symptoms of disease and the diagnosis is based on clinical, radiological and microbiological findings. IGRA, IFN-γ release assays; Mtb, *Mycobacterium tuberculosis*; TB, tuberculosis. [§] Data from a meta-analysis in adult population [66]; [#] data from a study in a low TB endemic country [67].

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