Point-of-care test for tuberculosis: a boon in diagnosis

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Abstract

Rapid diagnosis of tuberculosis (TB) is an effective measure to eradicate this infectious disease worldwide. Traditional methods for screening TB patients do not provide an immediate diagnosis and thus delay treatment. There is an urgent need for the early detection of TB through point-of-care tests (POCTs). Several POCTs are widely available at primary healthcare facilities that assist in TB screening. In addition to the currently used POCTs, advancements in technology have led to the discovery of newer methods that provide accurate and fast information independent of access to laboratory facilities. In the present article, the authors tried to include and describe the potential POCTs for screening TB in patients. Several molecular diagnostic tests, such as nucleic acid amplification tests, including GeneXpert and TB-loop-mediated isothermal amplification, are currently being used as POCTs. Besides these methods, the pathogenic component of *Mycobacterium tuberculosis* can also be utilized as a biomarker for screening purposes through immunological assays. Similarly, the host immune response to infection has also been utilized as a marker for the diagnosis of TB. These novel biomarkers might include Mtb85, interferon-γ inducible protein-10, volatile organic compounds, acute-phase proteins, *etc*. Radiological tests have also been observed as POCTs in the TB screening POCT panel. Various POCTs are performed on samples other than sputum, which further eases the screening process. These POCTs should not require large-scale manpower and infrastructure. Hence, POCT should be able to identify patients with *M. tuberculosis* infection at the primary healthcare level only. There are several other advanced techniques that have been proposed as future POCTs and have been discussed in the present article.

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Introduction

Point-of-care testing is referred to as a sensitive and specific bedside test for the diagnosis of a patient's disease, its monitoring, and its management. Tremendous advances in technology have led to the development of point-of-care diagnostics that aid in improved clinical outcomes. The point-of-care test (POCT) facilitates early medical decisions, effective patient triage in emergencies, reduced complications, and improved adherence to treatment [1]. POCTs for tuberculosis (TB) are particularly important in resource-limited settings [2]. The World Health Organization (WHO) has laid out criteria for an ideal POCT, which include affordability, sensitivity, specificity, user friendliness, equipment-freeness, robustness, rapidity, and finally being easily accessible and deliverable to end users [3]. POCT for infectious diseases allows healthcare providers to quickly initiate appropriate treatment. Among infectious diseases, even though TB has become a largely treatable disorder, it remains a major cause of death worldwide. The delay in diagnosis of TB makes infected individuals a central carrier of *Mycobacterium tuberculosis,* which has the potential to infect other individuals [4]. Therefore, the goal of the "End TB" strategy can be achieved by rapid detection of active TB cases using diagnostic facilities that are easily provided at health

care centers. Conventional methods of diagnosis, such as smear microscopy, have low sensitivity, drug resistance cannot be determined, and traditional culture methods are time-consuming. On the other hand, live and dead *M. tuberculosis* could not be differentiated by molecular methods. Also, immunological methods are being utilized to demarcate active and latent TB cases, but these tests do fail to some extent [5]. Therefore, to overcome several limitations of existing diagnostic modalities, various other methods have already been defined as POCTs, and in recent years, advanced, rapid new approaches have been in the pipeline of development. The present review discusses numerous diagnostic techniques that are either current POCTs or future potential candidates.

Methods

A comprehensive literature search was accomplished on electronic platforms such as PUBMED and Google Scholar for publications in English. The following key words were used, either individually or in combination: Point of care test for TB, molecular test, WHO-approved tests, advanced POCT, early diagnosis, POCT in LMICs. We excluded the publications that did not mention the use of diagnostic tests as a point of care. Key data from 81 articles were compiled and described in the present review.

Point-of-care tests used in the present period

Sputum smear microscopy

Sputum smear microscopy (SSM) has been the keystone in diagnosing *Mycobacterium* (Mtb) infection and is performed in peripheral microscopy centers, which are associated with primary health care centers providing TB therapy. It is the primary method of diagnosis in low- and middle-income countries. It is a very specific and beneficial technique in areas with a very high prevalence of TB [6]. This method utilizes Ziehl-Neelsen staining or fluorescent staining using Auramine-O/Auramine-rhodamine to detect acid-fast bacilli in sputum samples [7]. Even in the era of molecular diagnostics, smear microscopy is inexpensive, rapid, and substantially specific in diagnosis in highly prevalent areas. Despite these advantages, it has low sensitivity and cannot distinguish between live and dead Mtb and tubercular and non-tubercular Mtb [6]. Nowadays as well, SSM remains a major and primarily used method in clinical labs for the diagnosis of TB in resource-limited settings.

Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) can rapidly detect a small quantity of Mtb DNA with different modifications of polymerase chain reaction (PCR) amplification. In recent years, improvements in simplicity and automation have led to NAAT being identified as an attractive and potential candidate for POCT for TB. Laboratory-based diagnosis of TB is performed using several commercially available NAATs. Although the accuracy of the NAATs for respiratory samples is usually comparable, their inclusion in POCT relies basically on ease of use, rapidity of sample preparation and test completion, infrastructure, and cost-effectiveness [8]. One of the major disadvantages of NAATs is their inability to distinguish between live and dead TB bacilli. The Truenat assay is developed by MolBio Diagnostics Pvt. Ltd. (Verna, India). It is based on real-time PCR chips that are analyzed on the company's Truelab instruments. The instruments are compact, portable, and can be operated with a battery pack, making them ideal for use as a POCT [9]. Xpert *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) is a real-time quantitative PCR for Mtb that amplifies the *rpoB* gene-containing mutations responsible for rifampicin resistance. Thus, it can detect TB infection and RIF resistance simultaneously. There are several studies that have demonstrated Xpert to function as a POCT in well-resourced settings [10]. Advanced Xpert MTB/RIF Ultra has a better limit of detection of 16 CFU/mL in comparison to 114 CFU/mL of Xpert MTB/RIF [11]. GeneXpert OMNI is a small, portable POCT platform with a single standalone that has the potential to process Xpert cartridges in extreme conditions [12]. Similarly, other point-of-care NAATs are also under development, such as Q-point-of-care from QuantuMDx (Newcastle upon Tyne, UK) which is proposed to produce results in less than 30 minutes [13]. GeneXpert is the foremost used method for TB detection and provides rapid results in the current scenario. Recently, one of the studies conducted in a resource-limited setting showed the feasibility and sensitivity of GeneXpert in saliva samples of TB patients [14]. Although it deals with inadequate facilities and implementation barriers in low and low middle income countries (LMICs), GeneXpert remains a crucial point-of-care diagnostic platform for this infectious disease.

Loop-mediated isothermal amplification

TB-loop-mediated isothermal amplification (LAMP) is the WHO-endorsed molecular assay as an alternative to smear microscopy, which has a pooled sensitivity of 78% in clinical validation by the WHO [15,16]. LAMP is based on nucleic acid amplification through isothermal conditions. It involves autocycling, strand displacement DNA synthesis activity, and detection through visible turbidity in UV light [17]. TB-LAMP is comparatively easier, less labor-intensive, and has higher sensitivity and specificity as compared to smear microscopy. As LAMP does not require a thermocycler or fluorescence detection system, it has the potential to reduce test cost and time of diagnosis in peripheral lab or community settings. In comparison to other NAATs like Xpert MTB/RIF and point-of-care Xpert OMNI, which have better sensitivity and specificity, TB-LAMP does not provide additional benefits. Instead, it is being used in addition to the above NAATs, replacing only smear microscopy [15,18]. Therefore, in the absence of well-resourced infrastructure, TB-LAMP provides a better and more rapid platform for the diagnosis of TB at the point of care. Multiplex LAMP coupled with a fluorescent-based detection system has been developed to differentiate between Mtb and nontuberculous mycobacteria and thus show better performance than PCR or the traditional LAMP method. Several miniaturized LAMP techniques, such as microfluidic, electrochemical, paper-based, and digital methods, have been developed for rapid sample processing and screening of pathogens [19]. One of the studies evaluated the efficiency of the WHO-approved TB-LAMP kit in a sputum sample of TB patients in LMIC and indicated its potential to be used at microscopy centers in a resource-limited setting [20]. Similarly, another study demonstrated the good sensitivity and specificity of TB-LAMP for Mtb detection in LMICs [21].

Lipoarabinomannan assay

The diagnostic tests for TB infection mainly rely on sputum samples, which are difficult to obtain and have low sensitivity in children and immunocompromised people. The lipoarabinomannan (LAM) assay is the only WHO-recommended test to rapidly detect active TB in urine samples [22]. The cell envelope of *M.*

tuberculosis contains a LAM component that circulates in the blood after replicating Mtb degrades. LAM is filtered across the glomerular basement membrane of the kidneys into the urine. AlereLAM is the first commercially available lateral flow assay (LFA) for TB detection [23]. It is an immunochromatographic test for the qualitative detection of LAM antigen in human urine using specific antibodies against LAM. In addition to this, in recent years, Fujifilm TB LAM has been developed with a similar working principle and better sensitivity, even in HIV-infected TB patients [24]. These LAM assays have the potential to reach pediatric patients and adults regardless of HIV status and site of infection. As LAM has been observed to assist in the diagnosis of TB in immunocompromised individuals, there have been multiple studies that are being focused on the utilization of LAM biomarker assays even in paucibacillary diseases like miliary TB and lymph node TB [25-27]. Hence, urinary LAM has gained importance in the above-mentioned TB diagnostics. Therefore, urine LAM is the most prominent and promising diagnostic biomarker for use as a point-of-care TB test. The development of the lateral flow method has increased the utility of the LAM assay as a POCT, and as it can be easily done in urine samples, it proves to be advantageous over other tests that depend on sputum samples [22]. Due to modifications in the assay and the ease of obtaining samples, the LAM assay has been approved for TB diagnosis in LMICs.

Biosensors

These are analytical devices that utilize the principle of converting the biochemical reaction or interaction of isolated enzymes, receptor proteins, antibodies, whole cells, or tissue with a specific chemical compound into an optical, electrical, or thermal signal. The biosensors are classified into electrochemical, optical, mechanical, and magnetic based on the principle of the transducer [28]. They are also classified based on application as TB biosensors into electronic noses, nanowires, fiber-optics, breathalyzers, surface plasmon resonance, quartz crystal microbalances, magnetoelastics, diagnostic magnetic resonance, and magnetic barcodes. There are many advantages associated with the use of biosensing technologies, including: i) rapid and sensitive detection; ii) highly specific; iii) rapid response time; iv) capability to provide continuous data with minimal sample quantity requirements [29]. These biosensors are being developed using different omics approaches, such as genomics, proteomics, metabolomics, and lipidomics, to provide efficient, sensitive, and specific biosensors for TB diagnosis [30]. Besides several advancements, large-scale usage of biosensors needs to be promoted to include them in routine diagnostic modalities. In 2023, a research group developed a portable DNA electrochemical biosensor in one of the LMICs that can detect TB robustly, sensitively, and specifically *via* DNA hybridization with its *IS6110* gene marker, thus providing a potential PCOT in LMICs [31]. Hence, biosensors have great potential for PCOT in view of effective TB case finding.

Volatile organic compounds by breath analysis

Multiple signature molecules in breath can be detected using electric nose devices or captured and concentrated in a collection bag, which is later analyzed by gas chromatography or mass spectrometry [32]. One of the commercial products, *i.e.,* the TB breathalyzer, is being developed by Rapid Biosensor Systems (Cambridge, UK), allowing for portability and instant results with a simple breath test for TB. The reading and analysis of the sample take only a few minutes. Results from this non-invasive method correlate well with X-rays, sputum smears, and clinical exams and are unaffected by other conditions such as HIV, cancer, *etc*. Recently, Saktiawati *et al.* (2021) started a trial study for the application of the electric nose in TB detection and aimed at providing data concerning the sensitivity and specificity of the eNose-TB, time, and cost analysis of the screening algorithm with the eNose in a resource-limited set-up [33].

Point-of-care tests planned to be used in the future

Aptamers

They are target-specific single-stranded DNA or RNA detection molecules, which have higher sensitivity and specificity for target antigens. Structurally, they are small biomolecules ranging from 20 to 60 nucleotides and mimic antibodies. These are cost-effective, more stable at high temperatures, with better shell life and no variation among different batches (as with antibodies) [34]. Therefore, global efforts are being made to introduce aptamers in TB diagnostics and therapeutic monitoring. A previous study successfully raised aptamers to TB-specific antigens, which were detected in clinical samples [35]. Aptamers designed against the whole bacterium are suitable for diagnostic purposes as they can recognize different epitopes present in Mtb and detect them in human fluid, *i.e.,* blood, serum, and bronchoalveolar lavage. Aptamers are emerging as alternative molecules with superior properties to antibodies used in previous immunological assays. Aptamers against Mtb antigens such as culture filtrate protein-10 (CFP-10), early secretory antigenic (ESAT-6), and heterodimers of CFP-10-ESAT-6 have been identified and investigated for their capability of detecting active TB in clinical sputum samples [36,37]. A collaborative study developed two DNA-aptamer-based diagnostic tests, namely an aptamer-linked immobilized sorbent assay (Aptamer ALISA) and an electrochemical sensor in LMICs, for the direct detection of the TB biomarker HspX in sputum [35]. The application of aptamers as recognition elements in the LFAs can potentially lead to the development of point-of-care diagnostic devices.

Genome sequencing

It is a versatile tool for rapid and accurate detection of Mtb, which aids in better TB infection management through the successful determination of clinically significant mutations. There are various next-generation sequencing (NGS) methods, including whole genome sequencing [38], targeted NGS [39], and shotgun NGS [40], which are frequently used in the diagnosis of Mtb drug resistance. NGS is the most comprehensive molecular-based approach for TB detection. Sequencing of the whole cell genome provides more comprehensive genomic data. It allows for the identification of mutations, which further confers drug resistance to infecting Mtb organisms. On the other hand, targeted NGS provides faster and less information because it focuses on specific genomic areas for deeper analysis. Shotgun NGS determines the sequence of every chromosome and entire genome through random DNA fragments with overlapping ends [41]. The feasibility of these techniques can be attained in high-income and low-TB-burden countries. Therefore, these technologies are in their initial phases only in the diagnosis of TB and drug-resistant TB in current clinical settings. Besides advancements in technologies, the high cost of sequencing methods makes their utility in diagnosis a difficult task and makes them unsuitable for POCT in low- and middle-income countries.

Biomarker-pathogen and host

The target for the global control of TB disease demands the development of simpler and more accurate diagnostic tests. It requires specific TB diagnostic tests that are low-cost, minimally invasive, non-sputum-based, and highly sensitive, utilizing easily accessible biological specimens such as blood, saliva, urine, *etc*. [42]. Apart from Mtb DNA and cell wall LAM component detection, there are various other biomarkers from the perspective of the pathogen, such as the Ag85 complex [43], ESAT-6, CFP-10 [44], *etc*. There are several immune response biomarkers that are under the pipeline in the process of POCT development. C-reactive protein (CRP) is one such host immune biomarker of infection and inflammation. Although reliable POCTs for detecting CRP are widely available and CRP is a sensitive marker [45], it is a non-specific biomarker of infection and inflammation. Therefore, it can be included in the combined biomarker approach. Another possible biomarker that is being extensively studied with promising results is host immune response antibodies. A report suggested a direct correlation of TB-specific immunoglobulin G4 with disease activity [46]. A few cluster of differentiation markers are also being studied by flow cytometry and have attained specificity and sensitivity compatible with the target product profile (by WHO) for confirmatory tests [47]. One of the studies evaluated the host serum protein biomarkers of TB in LMICs and indicated complement factor H as the best suitable biomarker for functioning as point-of-care screening [48]. Although multiple studies have been performed in the discovery of TB-specific biomarkers, the potential biomarkers thus identified suffer several challenges, such as heterogenous population, variability in a cohort, laboratory variability, and costs. Various blood-based transcriptional biomarkers have also been proposed for the identification of incipient or active TB. A systematic review has shown that among the best-performing blood transcription biomarkers, *BATF2*, Kaforou25, Roe3, and Sweeney3 have equivalent diagnostic accuracy independent of HIV status. These biomarkers have attained the minimum criteria defined by the WHO for triage tests and not confirmatory tests [49]. Another report has demonstrated the role of four-transcript signatures in predicting TB progression [50]. One of the studies has identified the role of transcriptional biomarkers in treatment monitoring as multiple mRNA decline after treatment of TB [51]. However, ease of obtaining samples and their processing are some of the advantages, but equipment and complexity in the analysis of transcriptional signatures halt its usage as a POCT in resource-limited clinical settings. Recently, a research group assessed the utility of 3-gene transcriptomic signatures (*BAFT2*, *ETV7*, and *CD1C*) and displayed acceptable diagnostic performance in a resource-limited set-up [52]. Moreover, expression values of *RAB20* and *INSL3* genes in peripheral blood composed a biosignature that accurately classified TB status among patients with advanced HIV in two cohorts from LMICs [53]. Identification of several transcriptional biomarkers is still in the pipeline, and the development of a panel of markers is in the initial phases of discovery and analytical validation in multiple cohorts of TB patients both at national and international levels.

Artificial intelligence-based interpretation

Chest radiography is basically performed as a triage test for patients with typical symptoms of TB or TB-related risk factors. An increasing number of evidence shows that countries with high incidences of TB and low- or middle-income populations are utilizing mobile clinics to bring these technologies to high-risk populations [54]. Pre-screening with automated chest X-rays before Xpert (NAATs) is a promising TB point-of-care diagnostic in a resource-constraint set-up, as it could substantially reduce cost and increase daily throughput [55]. The computer-aided detection (CAD) products use artificial intelligence (AI) to indicate the likelihood of TB by analyzing radiographs and determining abnormal scores. The WHO has conditionally recommended the use of CAD as an alternative to human interpretation of digital chest X-rays for screening and triage tests of TB in patients with an age above or equal to 15 years [56]. The literature on AI applications for healthcare in LMICs has been steadily growing in recent years. A study evaluated the efficiency of CAD software for chest X-ray interpretation in the detection of TB and demonstrated that six CAD software programs [Qure.ai (Mumbai, India), DeepTek (Pune, India), Delft Imaging (‘s-Hertogenbosch, Netherlands) JF Healthcare (China), OXIPIT (Vilnius, Lithuania) and Lunit (Seoul, South Korea)] were working at par with expert readers (blinded clinicians) [57]. Researchers in different LMICs applied machine learning and signal processing methods to digital chest radiographs to identify TB cases [58] and drug-resistant TB cases [59]. Recently, a laboratory group from LMICs generated an algorithm for the application of AI in the monitoring of medication adherence for TB treatment [60]. Briefly, different types of POCT that are either currently being used or under pipeline have been represented in Figures 1 and 2. Some of the POCTs that have been included in the WHO guidelines for the rapid detection of TB are mentioned in Table 1.

Point-of-care modalities for diagnosing latent tuberculosis infection

In latent tuberculosis infection (LTBI), mycobacteria are not directly detectable and are hence measured *via* the host immune response against Mtb. For several years, the tuberculin skin test

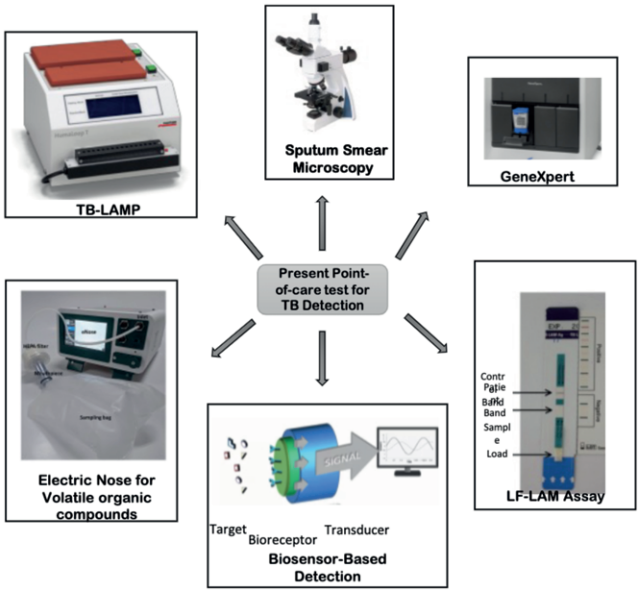

Figure 1. Representation of the currently used point-of-care tests for the diagnosis of tuberculosis. TB-LAMP, tuberculosis-loop-mediated isothermal amplification; TB, tuberculosis; LF-LAM, lateral-flow lipoarabinomannan.

(TST) has been the keystone test for LTBI diagnosis. Although this test is cost-effective and simple, there are several disadvantages, such as a double visit for the test, subjective interpretation, cross-reactivity in persons with BCG vaccination, and no assessment of immune anergy. Later, a specific immunodiagnostic test, *i.e., the* interferon (IFN)-γ release assay (IGRA), was developed. The cytokine IFN-γ is secreted by T-cells exposed to Mtb-specific antigens ESAT-6 and CFP-10 [61]. Moreover, a chemokine-inducible protein-10 (IP-10) has comparable diagnostic accuracy to IFN-γ and higher sensitivity in HIV-infected persons. Both techniques, TST and IGRA, had comparable abilities to predict short-term progression to active TB.

Interferon-γ release assay

IGRAs are *in vitro* blood tests of cell-mediated immune response that measure T cell release of IFN-γ following stimulation by antigens unique to *M. tuberculosis* (CFP-10 and ESAT-6) and a few other mycobacteria [62]. There are basically two types of assays used, including enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immune absorbent spot (ELISPOT). The basic protocol of the two methods is shown in Figure 3. There are three commercially available IGRAs, including the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay, the QuantiFERON-TB Gold Plus (QFT-Plus) assay (ELISA), and the tuberculosis-specific enzyme-linked immunospot (T-SPOT-TB) assay (ELISPOT). QFT-GIT contains long peptides derived from ESAT-6 and CFP-10, and QFT-Plus includes both these long peptides and shorter peptides in an additional tube to induce IFN-γ production. The inclusion of peptides for stimulation of CD8 T cells has been reported to improve the discrimination of LTBI from active TB. The QFT-Plus assay demonstrated a stronger association with increased Mtb exposure compared with QFT-GIT in adults with LTBI. Although both assays correlated well for LTBI diagnosis, the QFT-Plus exhibits a higher sensitivity with similar specificity regardless of age. T-SPOT. The TB assay also uses the Mtb antigens ESAT-6 and CFP-10 and quantifies the number of IFN-γ-producing T cells (spot-forming cells) [63]. Several attempts are being made to evaluate the role of QFT assays in POCT. One of the previous studies assessed the potential of LFA-based IGRA and demonstrated good diagnostic accuracy as compared to the QFT-GIT assay [64]. Recently, Miotto *et al.* (2022) studied the performance of the qualitative method of QFT-Plus with fluorescence lateral flow reader (QIAreach QFT) in the rapid detection of TB infection [65]. One of the studies conducted in LMICs has shown the utility of IGRAs in screening for LTBI sta-

Table 1. Point-of-care tests approved by the World Health Organization for diagnosing *Mycobacterium tuberculosis* (WHO 2022, update).

S. no.	Diagnostic platform		WHO approval status
1.	SSM		✓✓
2.	NAATs	TrueNat assay	✓
		Xpert MTB/RIF	✓
		Xpert MTB/RIF Utra	✓
3.	Lateral Flow-LAM assay		✓
4.	Chest X-ray		✓
5.	TB-LAMP		✓
6.	IGRA for LTBI		✓

SSM, sputum smear microscopy; NAATs, nucleic acid amplification tests; MTB/RIF, *Mycobacterium tuberculosis*/rifampicin; LAM, lipoarabinomannan; TB-LAMP, tuberculosis-loop-mediated isothermal amplification; IGRA, interferon-γ release assay; LTBI, latent tuberculosis infection; WHO, World Health Organization.

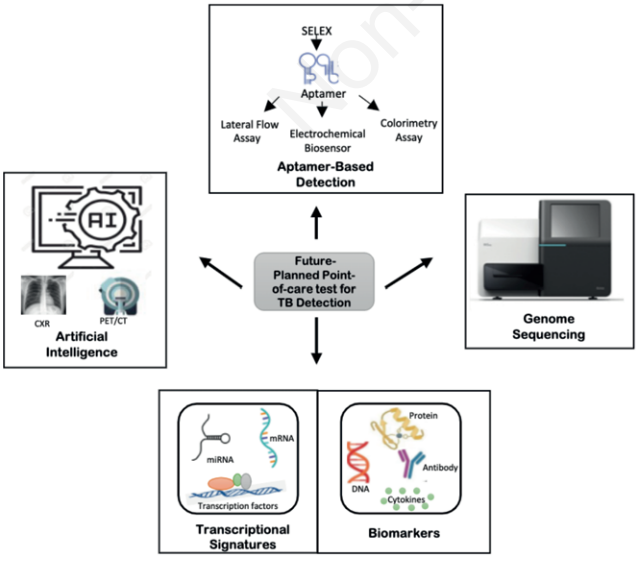

Figure 2. Illustration of a point-of-care test for tuberculosis diagnosis which is proposed to be used in the future. CXR, chest X-rays; PET/CT, positron emission tomography/computed tomography.

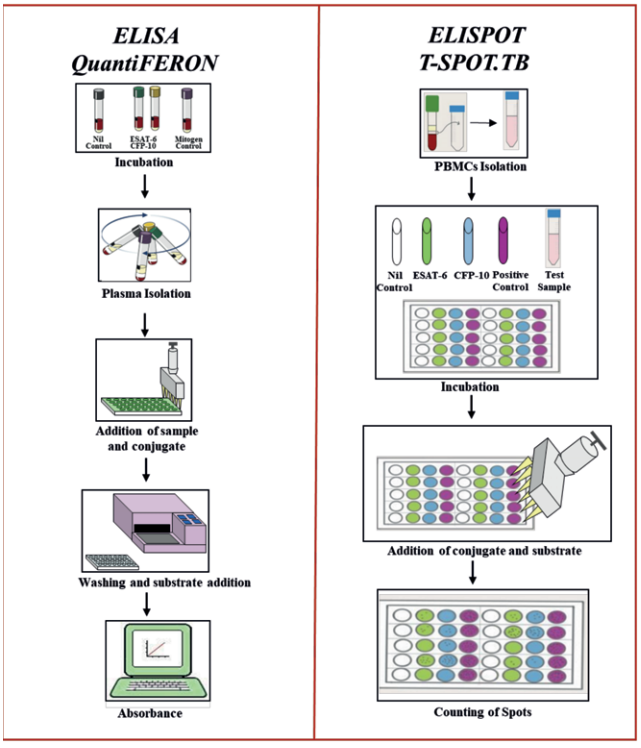

Figure 3. Depiction of interferon-γ release assay by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immune absorbent spot (ELISPOT) for detection of latent tuberculosis. PBMCs, peripheral blood mononuclear cells; CFP-10, culture filtrate protein-10; ESAT-6, early secretory antigenic.

tus conversion [66]. Although modified IGRA tests appear promising in discriminating between LTBI and active TB, they need further validation in both high- and low-risk populations.

Other immunological markers for latent tuberculosis infection detection

There are some targets or immunological markers that have been proposed for a long time to distinguish between LTBI and active TB. Analysis of cellular profile, CD4 T-cell subset secreting tumor necrosis factor (TNF)-α-only with effector memory cell phenotype CD45RA-CCR7-CD127, has been known to be associated with the progression of LTBI to active TB in an immunocompetent population [67,68]. Moreover, the cellular response against mycobacterial latency-associated antigens, *i.e.*, DosR has been important in identifying LTBI or active TB (ATB) individuals. On the other hand, the host immune response to different Mtb antigens, such as specific miRNA and molecular signatures, is important in blood transcriptome analysis [63]. However, these proposed potential candidates need to be validated in distinct populations with different risks of TB occurrence.

Cytokines as biomarker for tuberculosis diagnosis: potential for point-of-care test

New biomarkers are required to evaluate both pathogen and host key elements in response to infection. Identification of non-sputum-based biomarkers for predicting the risk of developing active TB and adequate responsiveness to treatment is an urgent need. Cytokines are key molecules that regulate immunological responses and have been extensively studied for their potential as diagnostic and prognostic biomarkers of TB. Numerous reports have highlighted the role of cytokines as biomarkers in TB infection reactivation, disease, and cure [69]. Interleukin (IL)-2, IP-10, IL-5, and IL-10 had promising diagnostic performance for TB infection, including both active TB and LTBI [70]. Some cytokines have been shown to have the potential to distinguish among patients with ATB and LTBI, such as macrophage inflammatory protein (MIP)-1β, TNF-α, IL-12p40, and IL-17. The concentrations of these multiple cytokines change significantly during TB treatment, and these levels are determined by multiplex immunoassays [71]. Thus, the Bio-plex multiplex assay allows simultaneous quantification of up to 500 proteins, peptides, and nucleic acid targets. Mycobacterial antigen-stimulated eight cytokines, *i.e.*, IL-1ra, IL-2, IL-10, IL-13, TNF-α, IFN-γ, IP-10, and MIP-1β, were significantly higher in TB-infected participants compared with TB-uninfected individuals, as measured by the Luminex multiplex immunoassay [72]. Similarly, the plasma cytokine signatures of TNFα, IL-2, and IL-17A demonstrated the potential of an accurate biomarker for the diagnosis of pediatric TB [73]. Several other studies have also shown various combinations of cytokine biomarkers proposed to have significance in predicting TB infection, as well as discrimination from LTBI and other sick controls. Additionally, in one of the studies, 12 biomarkers out of 20 host biomarker signatures were shown to have the capability of being a POCT for TB triage diagnosis [74]. Besides cytokines, multiplex assays are also used for other proteins such as CRP, serum amyloid A, serum amyloid P, Ferritin, *etc*. [75]. Recently, a report suggested a panel of chemokines identified by multiplex assay as a diagnostic biomarker for pediatric TB [76]. Therefore, the ease of multiplex immunoassay and its potential to screen multiple biomarkers in a single assay make it an ability to be included in POCT for TB infection and treatment outcome.

All the techniques with potential for POCT have been outlined in Figure 4. The basic categorization has been done to clearly indicate the group to which each method belongs.

Challenges to the development of point-of-care tests

Over the past decades, there have been several advances in the development of point-of-care, but those platforms may have limited accuracy and impact on TB diagnosis due to several barriers. The point-of-care suffers various technical challenges due to the complexities of the host and pathogen. The foremost reason includes the type of sample, sputum, which is scarce in HIV-infected children. Other factors found to be responsible might include antibody profiles in patients with ATB that overlap with LTBI or those with non-tubercular infection. The Mtb antigens can be expressed differentially in body compartments, and sometimes there is a lack of suitable antigenic targets. The development of POCT also confronts issues in test accuracy, which may vary in HIV-infected *versus* uninfected persons and those with pulmonary *versus* extrapulmonary TB. Also, HIV-infected people and those with compromised immunity may have colonization with non-tuberculosus mycobacteria, which might produce false results in them [77]. As a lateral flow format has been developed for various platforms, sensitivity is often suboptimal when using these assays. Previous qualitative research identified themes affecting POCTs, which included the main theme, *i.e.,* ″relationships" among

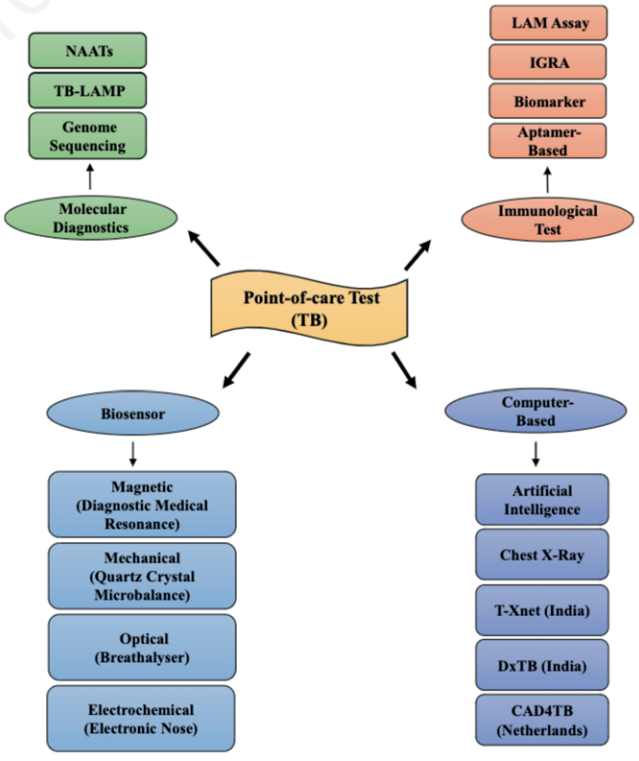

Figure 4. An overview of point-of-care tests for diagnosing tuberculosis infection. NAATs, nucleic acid amplification tests; TB-LAMP, tuberculosis-loop-mediated isothermal amplification; LAM, lipoarabinomannan; IGRA, interferon-γ release assay; TB, tuberculosis; CAD4TB, computer-aided detection "4" tuberculosis.

providers and between providers and patients, influenced by the cross-cutting theme of 'infrastructure [78]. Apart from these difficulties, there is a lack of information on the target product profile, a lack of funding for biomarker research, a lack of strong national regulatory frameworks, a lack of accountability, transparency, and commitment, a weak capacity of program managers to effect change, inadequate equipment and staff [79], and variable quality of diagnostic services and development of TB-point-of-care, which are preferably suitable for resource-rich settings [77]. Recently, a group of researchers reviewed POCT based on the two most common technologies, *i.e.*, LFA and NAATs. Some of the challenges with using LFA tests are: limitations in sensitivity; test accuracy relies on the quality and preparation of the antibodies; analysis time is dependent on the physical properties of the sample; and qualitative or semi-quantitative results are obtained. The challenges with using NAAT-based tests are complex and include bulky equipment for thermal cycling; higher power consumption and longer turnaround times; requirement of high temperatures of 95°C to denature double-stranded-DNA by different types of PCR (rolling circle amplification, strand displacement amplification, and nucleic acid sequence-based amplification); delay in results due to additional steps of DNA/RNA processing; dependence of the diagnostic performance of NAATs on type of sample used and presence of several amplification inhibitors in unprocessed samples; requirement of more than three primers in LAMP posing a high risk of primer dimer formation which can lead to false positives and undermine the accuracy of the POCT results; cost-effectiveness as a point-of-care; and limited multiplex LAMP assays [80]. Hence, much development in the field of TB-POC is still required to overcome the confrontations.

Conclusions

TB still represents a continuous challenge to worldwide public health. Although several factors contribute to TB control programs, diagnosis remains an important factor that needs attention. Significant advancements in research and development in the field of TB diagnostics have already been made. But the progressive growth of TB cases necessitates the development of POCT which are easily available or employed readily at primary healthcare facilities. Also, it is unlikely that a single POCT will be successful for all different kinds of populations and resource availability. However, a number of TB diagnostic tests that follow partially or fully the ideal conditions of POCT laid out by the WHO are currently being used, and several are in the pipeline. There are some techniques that are proposed to have the capability to be utilized as POCT in the distant future. As compared to conventional diagnostic platforms, NAATs have gained the most influential center of attention in the queue for developing POCT for TB. However, GeneXpert (cartridge-based amplification) delivers simultaneous detection of TB infection and drug resistance. Several modifications of native GeneXpert are being developed and studied for their utility as POCT. Although the point-of-care Xpert unit cost is higher than other tests, it is likely to offer good value for money relative to smear microscopy. TB-LAMP also offers good diagnostic potential as compared to conventional microscopy tests. On the other hand, the urine-LAM assay provides rapid bedside detection of TB. As LAM is a specific molecule of Mtb infection and a potent activator of the immune response, it is highly suitable to be used as POCT. Thus, the urinary LAM assay is an affordable and accessible diagnostic tool that could prove valuable in TB-endemic areas. Among the future proposed POCTs, NGS remains out of reach for most laboratories due to cost constraints and the requirement of skilled personnel and infrastructure in LMICs. Although multiple host and pathogen biomarkers are being identified and studied in the plasma/serum of TB or LTBI individuals, validation of these markers is required, along with cost analysis, to incorporate them into point-of-care modalities in high-TB-burden countries. Therefore, this review summarized the diagnostic potential of widespread techniques and biomarkers, along with their utility as POCT for TB.

References

1. Price CP. Point of care testing. BMJ 2001;322:1285-8.
2. Heidt B, Siqueira WF, Eersels K, et al. Point of care diagnostics in resource-limited settings: a review of the present and future of PoC in its most needed environment. Biosensors (Basel) 2020;10:133.
3. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting, 28-29 April 2014. Available from: https://apps.who.int/iris/handle/10665/135617.
4. Sreeramareddy CT, Panduru KV, Menten J, Van den Ende J. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. BMC Infect Dis 2009;9:91.
5. Nema V. Tuberculosis diagnostics: challenges and opportunities. Lung India 2012;29:259-66.
6. Das PK, Ganguly SB, Mandal B. Sputum smear microscopy in tuberculosis: it is still relevant in the era of molecular diagnosis when seen from the public health perspective. Biomed Biotechnol Res J 2019;3:77-9.
7. Ulukanligil M, Aslan G, Tasçi S. A comparative study on the different staining methods and number of specimens for the detection of acid fast bacilli. Mem Inst Oswaldo Cruz 2000;95:855-8.
8. Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia 2018;10:4.
9. Indian Council of Medical Research. India moving forward in research towards new tools for tuberculosis. Available from: https://www.tbonline.info/posts/2017/11/9/india-moving-forward-research-towards-new-tools-tb/.
10. Horne DJ, Kohli M, Zifodya JS, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2019;6:CD009593.
11. Chakravorty S, Simmons AM, Rowneki M, 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:e00812-7.
12. García-Elorriaga G, del Rey-Pineda G. Practical and laboratory diagnosis of tuberculosis: from sputum smear to molecular biology. Cham, Springer: 2015.
13. Ortega C, Wood R, Murton H, et al. Diagnosis of pulmonary tuberculosis by oral swab analysis (OSA): optimisation and development of non-sputum, point-of-care methods. Int J Tuber Lung Dis 2019;23:S211.
14. Byanyima P, Kaswabuli S, Musisi E, et al. Feasibility and sensitivity of saliva GeneXpert MTB/RIF Ultra for tuberculosis diagnosis in adults in Uganda. Microbiol Spectr 2022;10:e0086022.
15. World Health Organization. The use of loop-mediated isothermal amplification (TB- LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. 2016. Available from: https://apps.who.int/iris/handle/10665/249154.
16. Shete PB, Farr K, Strnad L, et al. Diagnostic accuracy of TB-

LAMP for pulmonary tuberculosis: a systematic review and meta-analysis. BMC Infect Dis 2019;19:268.
17. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 2009;15:62-9.
18. Deng Y, Duan YF, Gao SP, Wang JM. Comparison of LAMP, GeneXpert, mycobacterial culture, smear microscopy, TSPOT. TB, TBAg/PHA ratio for diagnosis of pulmonary tuberculosis. Curr Med Sci 2021;41:1023-8.
19. Garg N, Ahmad FJ, Kar S. Recent advances in loop-mediated isothermal amplification (LAMP) for rapid and efficient detection of pathogens. Curr Res Microb Sci 2022;3:100120.
20. Cheng S, Pheng SH, Heng S, et al. Evaluation of loopamp assay for the diagnosis of pulmonary tuberculosis in Cambodia. BioMed Res Int 2020;2020:6828043.
21. Yadav R, Daroch P, Gupta P, et al. Diagnostic accuracy of TB-LAMP assay in patients with pulmonary tuberculosis... a case-control study in northern India. Pulmonology 2022;28:449-53.
22. Bulterys MA, Wagner B, Redard-Jacot M, et al. Point-of-care urine LAM tests for tuberculosis diagnosis: a status update. J Clin Med 2019;9:111.
23. Songkhla MN, Tantipong H, Tongsai S, Angkasekwinai N. Lateral flow urine lipoarabinomannan assay for diagnosis of active tuberculosis in adults with human immunodeficiency virus infection: a prospective cohort study. Open Forum Infect Dis 2019;6:ofz132.
24. Li Z, Tong X, Liu S, et al. The value of FujiLAM in the diagnosis of tuberculosis: a systematic review and meta-analysis. Front Public Health 2021:9:757133.
25. Cox JA, Lukande RL, Kalungi S, et al. Is urinary lipoarabinomannan the result of renal tuberculosis? Assessment of the renal histology in an autopsy cohort of Ugandan HIV- infected adults. PLoS One 2015;10:e0123323.
26. Simieneh A, Tadesse M, Kebede W, et al. Combination of Xpert® MTB/RIF and DetermineTM TB-LAM Ag improves the diagnosis of extrapulmonary tuberculosis at Jimma University Medical Center, Oromia, Ethiopia. PLoS One 2022; 17:e0263172.
27. Shah M, Martinson NA, Chaisson RE, et al. Quantitative analysis of a urine-based assay for detection of lipoarabinomannan in patients with tuberculosis. J Clin Microbiol 2010;48:2972-4.
28. Zhou L, He X, He D, et al. Biosensing technologies for Mycobacterium tuberculosis detection: status and new developments. Clin Dev Immunol 2011;2011:193963.
29. Srivastava SK, Van Rijn CJ, Jongsma MA. Biosensor-based detection of tuberculosis. RSC Adv 2016;6:17759-71.
30. Joshi H, Kandari D, Maitra SS, Bhatnagar R. Biosensors for the detection of Mycobacterium tuberculosis: a comprehensive overview. Crit Rev Microbiol 2022;48:784-812.
31. Taufiq S, Waqar M, Sharif MN, Abbas SR. Towards portable rapid TB biosensor: detecting Mycobacterium tuberculosis in raw sputum samples using functionalized screen printed electrodes. Bioelectrochemistry 2023;150:108353.
32. Teixeira RC, Rodríguez M, de Romero NJ, et al. The potential of a portable, point-of- care electronic nose to diagnose tuberculosis. J Infect 2017;75:441-7.
33. Saktiawati AM, Triyana K, Wahyuningtias SD, et al. eNose-TB: a trial study protocol of electronic nose for tuberculosis screening in Indonesia. PLoS One 2021;16:e0249689.
34. Kulabhusan KP, Hussain B, Yüce M. Current perspectives on aptamers as diagnostic tools and therapeutic agents. Pharmaceutics 2020;12:646.
35. Lavania S, Das R, Dhiman A, et al. Aptamer-based TB antigen tests for the rapid diagnosis of pulmonary tuberculosis: potential utility in screening for tuberculosis. ACS Infect Dis 2018;4: 1718-26.
36. Russell TM, Green LS, Rice T, et al. Potential of high-affinity, slow off-rate modified aptamer reagents for Mycobacterium tuberculosis proteins as tools for infection models and diagnostic applications. J Clin Microbiol 2017;55:3072-88.
37. Tang XL, Zhou YX, Wu SM, et al. CFP10 and ESAT6 aptamers as effective Mycobacterial antigen diagnostic reagents. J Infect 2014;69:569-80.
38. Pankhurst LJ, del Ojo Elias C, Votintseva AA, et al. Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. Lancet Respir Med 2016;4:49-58.
39. Colman RE, Mace A, Seifert M, et al. Whole-genome and targeted sequencing of drug- resistant Mycobacterium tuberculosis on the iSeq100 and MiSeq: a performance, ease- of-use, and cost evaluation. PLoS Med 2019;16:e1002794.
40. Messah AD, Pawitan JA. Next generation sequencing as rapid diagnosis of multidrug resistance tuberculosis. Adv Life Sci 2020;8:30-7.
41. Dookie N, Khan A, Padayatchi N, Naidoo K. Application of next generation sequencing for diagnosis and clinical management of drug-resistant tuberculosis: updates on recent developments in the field. Front Microbiol 2022;13:775030.
42. Goletti D, Petruccioli E, Joosten SA, Ottenhoff TH. Tuberculosis biomarkers: from diagnosis to protection. Infect Dis Rep 2016;8:6568.
43. Kashyap RS, Rajan AN, Ramteke SS, et al. Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study. BMC Infect Dis 2007;7:74.
44. Rodríguez-Hernández E, Quintas-Granados LI, Flores-Villalva S, et al. Application of antigenic biomarkers for Mycobacterium tuberculosis. J Zhejiang Univ Sci B 2020;21:856-70.
45. Yoon C, Semitala FC, Atuhumuza E, et al. Point-of-care C-reactive protein-based tuberculosis screening for people living with HIV: a diagnostic accuracy study. Lancet Infect Dis 2017;17: 1285-92.
46. Grace PS, Dolatshahi S, Lu LL, et al. Antibody subclass and glycosylation shift following effective TB treatment. Front Immunol 2021;12:679973.
47. Acharya MP, Pradeep SP, Murthy VS, et al. CD38+ CD27– TNF-α+ on Mtb-specific CD4+ T cells is a robust biomarker for tuberculosis diagnosis. Clin Infect Dis 2021;73:793-801.
48. Morris TC, Hoggart CJ, Chegou NN, et al. Evaluation of host serum protein biomarkers of tuberculosis in sub-Saharan Africa. Front Immunol 2021;12:639174.
49. Turner CT, Gupta RK, Tsaliki E, et al. Blood transcriptional biomarkers for active pulmonary tuberculosis in a high-burden setting: a prospective, observational, diagnostic accuracy study. Lancet Respir Med 2020;8:407-19.
50. Suliman S, Thompson EG, Sutherland J, et al. Four-gene pan-African blood signature predicts progression to tuberculosis. Am J Respir Crit Care Med 2018;197:1198-208.
51. Cliff JM, Lee JS, Constantinou N, et al. Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. J Infect Dis 2013;207:18-29.
52. Chendi BH, Jooste T, Scriba TJ, et al. Utility of a three-gene transcriptomic signature in the diagnosis of tuberculosis in a low-endemic hospital setting. Infect Dis 2023;55:44- 54.
53. Kulkarni V, Queiroz AT, Sangle S, et al. A two-gene signature for

tuberculosis diagnosis in persons with advanced HIV. Front Immunol 2021;12:631165.
54. Binepal G, Agarwal P, Kaur N, et al. Screening difficult-to-reach populations for tuberculosis using a mobile medical unit, Punjab India. Public Health Action 2015;5:241- 5.
55. Philipsen RH, Sánchez CI, Maduskar P, et al. Automated chest-radiography as a triage for Xpert testing in resource-constrained settings: a prospective study of diagnostic accuracy and costs. Sci Rep 2015;5:12215.
56. World Health Organization. WHO consolidated guidelines on tuberculosis: module 2: screening: systematic screening for tuberculosis disease. Available from: https://www.who.int/publications/i/item/9789240022676.
57. Codlin AJ, Dao TP, Vo LN, et al. Independent evaluation of 12 artificial intelligence solutions for the detection of tuberculosis. Sci Rep 2021;11:23895.
58. Lopes UK, Valiati JF. Pre-trained convolutional neural networks as feature extractors for tuberculosis detection. Comput Biol Med 2017;89:135-43.
59. Jaeger S, Juarez-Espinosa OH, Candemir S, et al. Detecting drug-resistant tuberculosis in chest radiographs. Int J Comput Assist Radiol Surg 2018;13:1915-25.
60. Sekandi JN, Shi W, Zhu R, et al. Application of artificial intelligence to the monitoring of medication adherence for tuberculosis treatment in Africa: algorithm development and validation. JMIR AI 2023;2:e40167.
61. Zellweger JP, Sotgiu G, Corradi M, Durando P. The diagnosis of latent tuberculosis infection (LTBI): currently available tests, future developments, and perspectives to eliminate tuberculosis (TB). Med Lav 2020;111:170-83.
62. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev 2014;27:3-20.
63. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres M. Diagnosis for latent tuberculosis infection: new alternatives. Front Immunol 2020;11:2006.
64. Hur YG, Hong JY, Choi DH, et al. A feasibility study for diagnosis of latent tuberculosis infection using an IGRA Point-of-care platform in South Korea. Yonsei Med J 2019;60:375-80.
65. Miotto P, Goletti D, Petrone L. Making IGRA testing easier: first performance report of QIAreach QFT for tuberculosis infection diagnosis. Pulmonology 2022;28:4-5.
66. Girish S, Kinikar A, Pardesh G, et al. Utility of the interferon-gamma release assay for latent tuberculosis infection screening among Indian health-care workers. Indian J Commun Med 2021;46:281-4.
67. Morgan J, Muskat K, Tippalagama R, et al. Classical CD4 T cells as the cornerstone of antimycobacterial immunity. Immunol Rev 2021;301:10-29.
68. Halliday A, Whitworth H, Kottoor SH, et al. Stratification of latent mycobacterium tuberculosis infection by cellular immune profiling. J Infect Dis 2017;215:1480-7.
69. Mihret A, Abebe M. Cytokines and chemokines as biomarkers of tuberculosis. J Mycobac Dis 2013;3:2.
70. Wei Z, Li Y, Wei C, et al. The meta-analysis for ideal cytokines to distinguish the latent and active TB infection. BMC Pulm Med 2020;20:248.
71. Walzl G, Haks MC, Joosten SA, et al. Clinical immunology and multiplex biomarkers of human tuberculosis. Cold Spring Harb Perspect Med 2015;5:a018515.
72. Clifford V, Tebruegge M, Zufferey C, et al. Cytokine biomarkers for the diagnosis of tuberculosis infection and disease in adults in a low prevalence setting. Tuberculosis (Edinb) 2019; 114:91-102.
73. Kumar NP, Hissar S, Thiruvengadam K, et al. Discovery and validation of a three- cytokine plasma signature as a biomarker for diagnosis of pediatric tuberculosis. Front Immunol 2021;12:653898.
74. Mutavhatsindi H, Van der Spuy GD, Malherbe ST, et al. Validation and optimization of host immunological bio-signatures for a point-of-care test for TB disease. Front Immunol 2021;12:607827.
75. Chegou NN, Sutherland JS, Malherbe S, et al. Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax 2016;71:785-94.
76. Kumar NP, Hissar S, Thiruvengadam K, et al. Plasma chemokines as immune biomarkers for diagnosis of pediatric tuberculosis. BMC Infect Dis 2021;21:1055.
77. Dheda K, Ruhwald M, Theron G, et al. Point-of-care diagnosis of tuberculosis: past, present and future. Respirology 2013;18:217-32.
78. Engel N, Ganesh G, Patil M, et al. Barriers to point-of-care testing in India: results from qualitative research across different settings, users and major diseases. PLoS One 2015;10: e0135112.
79. Schito M, Peter TF, Cavanaugh S, et al. Opportunities and challenges for cost-efficient implementation of new point-of-care diagnostics for HIV and tuberculosis. J Infect Dis 2012; 205: S169-80.
80. Sachdeva S, Davis RW, Saha AK. Microfluidic point-of-care testing: commercial landscape and future directions. Front Bioeng Biotechnol 2021;8:602659.