Tests for tuberculosis infection: landscape analysis

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New and emerging tests for tuberculosis infection have the potential to improve accuracy, operational characteristics and end-user access. Evaluation of these tests in a standardised design would facilitate their endorsement and programmatic scale-up. https://bit.ly/327RBky

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Abstract

Background Only the tuberculin skin test (TST) and two interferon-γ release assays (IGRAs), QuantiFERON-TB Gold In-Tube and T-SPOT.TB, are currently endorsed by the World Health Organization as tests for tuberculosis (TB) infection. While IGRAs are more specific than the TST, they require sophisticated laboratory infrastructure and are costly to perform. However, both types of tests have limited performance to predict development of active TB. Tests with improved predictive performance and operational characteristics are needed.

Methods We reviewed the current landscape of tests for TB infection identified through a web-based survey targeting diagnostic manufacturers globally.

Results We identified 20 tests for TB infection: 15 *in vitro* tests and five skin tests. 13 of the *in vitro* tests are whole-blood IGRAs and 14 use early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), with or without additional antigens. 10 of the tests are based on assays other than an ELISA, such as a fluorescent lateral flow assay that requires less manual operation and shorter assay time and hence is more suitable for decentralisation compared with the existing IGRAs. Four of the five skin tests use ESAT-6 and CFP-10 proteins, while the remaining test uses a new antigen that is specific to *Mycobacterium tuberculosis* complex.

Conclusions New tests have the potential to improve accuracy, operational characteristics and end-user access to tests for TB infection. However, published data in various populations and settings are limited for most new tests. Evaluation of these new tests in a standardised design would facilitate their endorsement and programmatic scale-up.

Introduction

Tuberculosis (TB) remains the top cause of death from a single infectious disease agent worldwide [1]. The World Health Organization (WHO) set ambitious targets of reducing 2015 estimates for TB incidence by 90% and deaths by 95% by 2035 [2]. Treatment of TB infection to halt progression to disease, also known as TB preventive treatment (TPT), is one of the critical strategies to achieve the End TB Strategy targets. At the first United Nations High Level Meeting on TB in 2018, Member States committed to provide TPT to at least 30 million people by 2022: 6 million people living with HIV (PLHIV), 4 million children <5 years who are household contacts of people with TB and 20 million other household contacts [3]. The Stop TB Partnership's Global Plan to End TB (2018–2022) adapted the same targets, thus reaffirming the global commitment to scale-up TPT [4].

The uptake of TPT has been very slow. While various barriers exist, inaccessibility of tests for TB infection is commonly cited by national TB programmes as a major barrier to providing TPT [5, 6].

Programmatic implementation of current tests for TB infection is fraught with difficulties. Manufacturing challenges in the tuberculin skin test (TST) have led to periodic shortages [7], and access is hampered by the requirement to maintain the cold-chain for transportation and storage. High cost and inadequate laboratory infrastructure make it difficult to implement the alternative test for infection, the interferon-γ release assay (IGRA), in peripheral facilities or at the community level, especially in low- and middle-income countries. Moreover, existing tests for TB infection, the TST and IGRA, which measure immune response to stimulation by *Mycobacterium tuberculosis* antigens, have very low performance to predict development of active TB [8]. Development of new tests with improved predictive value is a high priority [9].

Partly as a result of this inaccessibility of tests and limitations in the accuracy and predictive performance for subsequent TB, tests for TB infection are currently not required before starting TPT in PLHIV and household contacts <5 years of age who reside in high TB burden countries [10]. However, for people in other at-risk populations, tests for TB infection are recommended to identify those who would benefit most from treatment to avoid unnecessary medication and risk of drug adverse events. There is thus a strong imperative to increase accessibility to tests for TB infection globally. Furthermore, even in PLHIV and child contacts, tests that are highly predictive of TB and easy to implement might enable better targeting of TPT. This calls for new tests with improved diagnostic performance and operational characteristics. For example, instrument-free tests or tests that can be performed with small, portable or hand-held, battery-operated instruments will allow deployment of tests at the lowest level of healthcare. Rapid tests (*e.g.* <1 h for results) would enable the diagnosis and initiation of treatment on the same day and facilitate uptake.

New tests for TB infection are starting to emerge. It is important to review the landscape of such tests and identify gaps in the pipeline to facilitate development and assay uptake. We conducted a landscape analysis of tests for TB infection. The aim of this review is to summarise tests for TB infection on the market and in the pipeline, and to highlight gaps and priorities.

Tests for new TB infection were identified through an online survey targeting diagnostic test manufacturers globally. We prepared the survey in English and piloted it with two manufacturers to assess clarity and relevancy of questions. The final version of the survey was posted online from 29 June 2020 to 15 July 2020. The launch of the survey was announced by the Foundation for Innovative New Diagnostics (FIND) and Stop TB Partnership, and was disseminated through their webpages, social media and list serves. We also directly invited test manufacturers that were known to FIND. The survey tool consisted of questions about specifications of the test (*e.g.* type of test, readout and antigens), operational characteristics, status of validation against commercially available tests and development stage. We obtained package inserts or equivalent if available. We also reviewed tests whose information was obtained through FIND's technology scouting activities and manufacturer interactions outside of this project. Results were analysed qualitatively. Tests for TB infection defined as those that measure immune response to stimulation by *M. tuberculosis* antigens and are intended for identifying individuals to be given treatment for TB infection were included in the review.

13 manufacturers participated in the survey, providing information on 14 tests for TB infection (11 *in vitro* tests and three skin tests) and one test that we considered as a test for incipient TB and was excluded from the rest of the review. Additionally, we identified four *in vitro* tests and two skin tests identified through the aforementioned other activities (table 1). In total, 20 tests for TB infection were reviewed.

We first summarise tests for TB infection currently endorsed by the WHO and then we describe tests new to the pipeline.

Tests for TB infection currently recommended by the WHO

The TST, also known as the Mantoux test, uses purified protein derivative (PPD), a mixture of antigens obtained from *M. tuberculosis*. Intradermal injection of PPD induces a delayed-type hypersensitivity reaction and the diameter of the induration is measured in millimetres 48–72 h after injection. The TST is affected by cross-reactions with the bacille Calmette–Guérin (BCG) vaccine and nontuberculous mycobacteria (NTM) as PPD contains proteins found in most mycobacterial species [11]. The impact of BCG on the TST reaction depends on the timing and frequency of BCG given. It is considered that BCG given at birth, which is the case in most high TB burden countries, affects adolescent and adult TSTs minimally [12]. Likewise, the proportion of false-positive results attributable to NTM is considered small. In a systematic review, the prevalence of false-positive TST results due to NTM was estimated to range from 0.1% to 2.3% across various settings [12]. The test is less sensitive in immunocompromised patients,

TABLE 1 List of all manufacturers and tests identified

Type	Survey response	Company	Country	Name of test
***In vitro* test**	Yes	bioMérieux	France	VIDAS TB-IGRA
	Yes	Boditech Med	Republic of Korea	ichroma IGRA-TB
	Yes	Erythra	USA	Erythra TB test
	Yes	Glory Biotechnologies	Republic of Korea	GBTsol Latent TB Test Kit
	No	LG Chem	Republic of Korea	AdvanSure I3 TB-IGRA; AdvanSure TB-IGRA
	Yes	LIONEX Diagnostics & Therapeutics	Germany	LIOFeron TB/LTBI
	Yes	Oxford Immunotec	UK	T-SPOT.TB
	Yes	QIAGEN	The Netherlands	QuantiFERON-TB Gold Plus; QIAreach QuantiFERON-TB Gold Plus; LIAISON QuantiFERON-TB Gold Plus
	Yes	QuantuMDx	UK	Unspecified (correlate of risk[#])
	Yes	R-Biopharm	Germany	IP-10 IGRA lateral flow; IP-10 IGRA ELISA
	Yes	SD Biosensor	Republic of Korea	STANDARD E TB-Feron ELISA; STANDARD F TB-Feron FIA (IFN-gamma)
Skin test	Yes	Anhui Zhifei Longcom Biopharmaceutical	China	EC-Test[¶]
	Yes	JSC Generium	Russian Federation	Diaskintest
	Yes	Serum Institute of India	India	C-Tb
	No	Zhejiang Hisun Pharmaceutical	China	Identification Allergen
	No	HDT Bio	USA	DPPD

[#]: this test was deemed a test for incipient tuberculosis; [¶]: recently renamed as C-TST.

e.g. those taking immunosuppressive agents, and PLHIV [13]. Because of these multiple factors affecting the TST reaction, the cut-off usually varies depending on history of BCG vaccination, prevalence of NTMs, presence of conditions impairing immunity, *etc.* [13].

Advantages of the TST include not requiring laboratory infrastructure or technicians and its low cost. Unlike IGRAs, phlebotomy is not necessary. However, administration of the TST and interpretation of skin induration requires training, and standardising administration and reading, and ensuring their quality is a challenge. The need for a return visit to read results increases barriers to patients. A cold-chain is required for transportation and storage of PPD.

Several PPD products are available, of which PPD-S2 (Aplisol; JHP Pharmaceuticals, Rochester, NY, USA and Tubersol; Sanofi Pasteur, Swiftwater, PA, USA) and PPD RT23 (AJ Vaccines, Copenhagen, Denmark) are used widely [11]. The potency of the standard dose of PPD RT23 and PPD-S2 is considered equivalent; however, PPD standardised against these products may not be available in some countries [11].

IGRAs are *in vitro* blood tests that measure the cellular immune response by quantitatively or qualitatively detecting IFN-γ release following stimulation by antigens specific to *M. tuberculosis*. In 2011, the WHO reviewed the evidence on the performance of two types of IGRA: QuantiFERON-TB Gold In-Tube (QFT-GIT) (QIAGEN, Venlo, The Netherlands) and T-SPOT.TB (Oxford Immunotec, Abingdon, UK) [14]. The review did not find a significant difference in predictive performance for the development of active TB between the IGRA and TST. In light of logistic challenges associated with the IGRA, the WHO did not recommend its use in low- and middle-income countries. However, the WHO updated the recommendation in 2018, recognising the global shortage of the TST, and now recommends both the IGRA and TST in all settings [10]. QFT-GIT is an ELISA-based whole-blood test that uses a peptide form of antigens specific to *M. tuberculosis*, *i.e.* early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), encoded by the RD1 gene, as well as TB7.7 (Rv2654c). The level of IFN-γ elicited by these antigens is quantified by ELISA. Recently, QuantiFERON-TB Gold Plus (QFT-Plus), the fourth generation of the QuantiFERON assay, has replaced QFT-GIT. QFT-Plus added an extra blood collection tube to measure both CD4 and CD8 T-cell responses to CFP-10 and ESAT-6 antigen stimulation.

Theoretical advantages include improved sensitivity in PLHIV and children as well as association with recent infection, leading to improved predictive performance [15]. However, evidence on its superiority over QFT-GIT is still limited. A recent review found comparable sensitivity of QFT-Plus and QFT-GIT in people with active TB as well as excellent agreement in high-risk groups, including contacts, immigrants, healthcare workers and immunocompromised patients [15]. A recently published study reported that HIV status or CD4 cell count did not significantly affect IFN-γ levels due to retention of a CD8-specific response [16]. Data on its predictive value are available from only one study. In a prospective study among TB contacts, 5.7% developed TB over 2 years, and the predictive performance was similar to that reported in T-SPOT.TB and QFT-GIT [17].

T-SPOT.TB measures the number of peripheral mononuclear cells that produce IFN-γ in response to ESAT-6 and CFP-10 by the ELISPOT assay. The laboratory procedure is more complex for T-SPOT.TB than QFT. The use of T-Cell Xtend reagents enables isolation of lymphocytes for up to 32 h (in contrast to 8 h without the reagents) after blood collection [18, 19]. A new reagent kit, T-Cell Select, is claimed to extend the storage for up to 54 h before sample processing; however, we could not find published validation studies. Both T-SPOT.TB and QFT-Plus require laboratory set-up and are more expensive than the TST. However, because these antigens are not present in most NTMs (thereby excluding detection of sensitisation to BCG strains and those NTMs other than *Mycobacterium marinum*, *Mycobacterium kansasii*, *Mycobacterium szulgai* and *Mycobacterium flavescens*), both QFT-Plus and T-SPOT.TB have higher specificity than TST [20].

New tests for TB infection

Figure 1 shows tests for TB infection in the pipeline, which are summarised below.

There are 13 *in vitro* tests for TB infection that are in the pipeline or commercialised but not endorsed by the WHO, 12 of which are whole-blood IGRAs and one, the GBTsol Latent TB Test Kit, uses a novel patented technology described later. Additionally, there are five new skin tests in the pipeline.

In vitro *tests for TB infection*

Tables 2 and 3 summarise the characteristics of *in vitro* tests for TB infection.

Recently, QIAGEN launched LIAISON QuantiFERON-TB Gold Plus. With the LIAISON XL analyser (DiaSorin, Saluggia, Italy), quantification of IFN-γ is performed automatically through a chemiluminescence immunoassay. This significantly reduces manual hands-on time and increases throughput; up to 25 tests can be performed per hour. Thus, LIAISON QuantiFERON-TB Gold Plus is optimal in high-throughput laboratories. The test has been validated against the standard QFT-Plus assay [21], and is commercially available in the European Union (EU) and USA.

Other ELISA-based whole-blood IGRAs include STANDARD E TB-Feron (SD Biosensor, Suwon, Republic of Korea), AdvanSure TB-IGRA ELISA (LG Chem, Seoul, Republic of Korea) and LIOFeron TB/LTBI (LIONEX Diagnostics & Therapeutics, Braunschweig, Germany), all of which are commercially available. The technological principle of these tests and operational characteristics are similar to QFT-GIT technology.

STANDARD E TB-Feron requires three tubes containing recombinant whole proteins of ESAT-6, CFP-10 and TB7.7, in contrast to peptide antigens used for QuantiFERON. In a study in healthcare workers in a tertiary hospital in the Republic of Korea who were tested for TB infection as part of an annual screening programme (n=425), the concordance rate between QFT-GIT and STANDARD E TB-Feron was 95.3% (κ=0.78) [22]. There are no published data on its agreement with WHO-endorsed IGRAs in other populations or its accuracy in people with active TB.

LIOFeron TB/LTBI uses four tubes, of which two tubes contain *M. tuberculosis*-specific antigens. One of the tubes contains recombinant fusion proteins of three antigens (ESAT-6, CFP-10 and TB7.7) included in QFT-GIT. In addition, the other tube includes alanine dehydrogenase antigen containing CD8 epitopes. There is only one published study on its performance. In a study in Italy, sensitivity in active TB patients (n=66) was 90% for LIOFeron TB/LTBI and 98% for QFT-Plus; specificity in healthy participants (n=151) was 98% and 97%, respectively [23].

AdvanSure TB-IGRA ELISA uses a peptide form of ESAT-6 and CFP-10 antigens. The test is commercially available. We have little information on this test, as the company did not participate in the survey.

	Early development	Clinical and laboratory validation	Regulatory	Commercially available
IGRA		AdvanSure I3 TB-IGRA[#] LG Chem, Republic of Korea Erythra TB test Erythra, USA IP-10 IGRA ELISA[#] R-Biopharm, Germany IP-10 IGRA lateral flow assay[#] R-Biopharm, Germany QIAreach QuantiFERON-TB QIAGEN, The Netherlands VIDAS TB-IGRA bioMérieux, France		AdvanSure TB-IGRA LG Chem, Republic of Korea ichroma IGRA-TB Boditech Med, Republic of Korea LIAISON QuantiFERON-TB Gold Plus QIAGEN, The Netherlands LIOFeron TB/LTBI LIONEX Diagnostics & Therapeutics, Germany STANDARD E TB-Feron ELISA SD Biosensor, Republic of Korea STANDARD F TB-Feron FIA (IGN-gamma) SD Biosensor, Republic of Korea T-SPOT.TB Oxford Immunotec, UK QuantiFERON-TB Gold Plus QIAGEN, The Netherlands
Other *in vitro* test	GBTsol Latent TB Test Kit Glory Biotechnologies, Republic of Korea			
Specific skin test		DPPD[#] HDT Bio, USA Identification Allergen[#] Zhejiang Hisun Pharmaceutical, China	C-Tb Serum Institute of India, India	Diaskintest JSC Generium, Russian Federation EC-Test[¶] Anhui Zhifei Longcom Biopharmaceutical, China
TST				RT23: AJ Vaccines/Statens Serum Institut, Denmark Laboratorio Nacional de Salud, Mexico Celltech Pharma, Spain PPD-S2: Tubersol; Sanofi Pasteur, USA Aplisol; JHP Pharmaceuticals, USA PPD-s; Nihon BCG Seizo, Japan PPD; SPAN Diagnostics/Arkray Healthcare, India PPD; Beijing Sanroad Biological Products, China

FIGURE 1 Tests for tuberculosis (TB) infection in the pipeline: at-a-glance. IGRA: interferon-γ release assay; TST: tuberculin skin test. [#]: tests whose manufacturer did not participate in the survey; [¶]: recently renamed as C-TST.

Simpler operation, faster results and closer to patients

Simplified versions of IGRAs are emerging, including QIAreach QuantiFERON-TB (QIAreach) (QIAGEN), STANDARD F TB-Feron FIA (IFN-gamma) (SD Biosensor), ichroma IGRA-TB (Boditech Med, Chuncheon, Republic of Korea) and AdvanSure I3 TB-IGRA (LG Chem). These tests require less manual handling than ELISA-based IGRAs and the results are available in 15–20 min once the 16–24 h incubation is completed. QIAreach uses the same antigens as QFT-Plus but requires only a single tube. A qualitative result, expressed as positive or negative according to the internal algorithm, is obtained by a fluorescence lateral flow reader. The reader, called an e-hub, is battery operable, can be connected to laboratory information management systems and can operate for 8 h on the battery supply. QIAreach is simple to use without the need for highly trained personnel; hence, it can decentralise testing for TB infection. Validation against existing tests for TB infection is currently ongoing. QIAGEN estimates the launch in 2021, with the initiation launch planned in Kenya, Nigeria, South Africa, Uganda and Zambia.

ichroma IGRA-TB is a fluorescence lateral flow immunoassay using CFP-10 and ESAT-6 peptide antigens. Two types of fluorescence readers are available. 1) ichroma II is a portable fluorescence reader with battery options, which provide results for a single test in 15 min. 2) ichroma 50 enables automation and three tubes can be directly loaded on the platform without a need for transfer of samples. It can process up to 60 tests per hour and thus it is suitable for laboratories that receive a large number of

TABLE 2 Characteristics of existing and new ELISA or ELISPOT-based interferon (IFN)-γ release assays (IGRAs)

	AdvanSure TB	IP-10 ELISA	LIOFeron TB/LTBI	QFT-Plus	STANDARD E TB-Feron	T-SPOT.TB
Type	WB IGRA	WB IGRA	WB IGRA	WB IGRA	WB IGRA	PBMC IGRA
Antigens	EC peptides	EC peptides	EC, TB7.7, ADH antigen (fusion protein)	EC peptides	EC, TB7.7 protein	EC peptides
Readout	ELISA	ELISA	ELISA	ELISA	ELISA	ELISPOT
Marker	IFN-γ	IP-10	IFN-γ	IFN-γ	IFN-γ	IFN-γ
Sample collection	3 tubes	3 tubes	1 heparin tube and distribute into 4 tubes	4 specialised tubes or a heparin tube and distribute	3 specialised tubes or a heparin tube and distribute	1 heparin tube or a specialised tube (Vacutainer CPT)
Interval before sample processing	No information	No information	Within 16 h	Within 16 h or 48 h at 2–8°C if drawn into heparin tubes	Within 16 h	Within 8 h, 32 h with T Cell Xtend or 54 h with T Cell Select
Incubation time	16–24 h	16–24 h	16–24 h	16–24 h	16–24 h	16–24 h
Assay time[#]	2 h	20 min	2.5 h	3 h	1.5 h	4 h
Regulatory approval	CE	CE	CE	CE, FDA	CE	CE, FDA

WB: whole blood; PBMC: peripheral blood mononuclear cell; EC: early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10); ADH: alanine dehydrogenase; IP-10: IFN-γ-induced protein 10; CE: European conformity mark; FDA: Food and Drug Administration. [#]: time from post-incubation to results.

samples. This test is based on a similar technological principle to other IGRAs, and requires phlebotomy and incubation. In a study in 60 healthy individuals including 10 TB contacts in the Republic of Korea, ichroma IGRA-TB using the ichroma II reader had high agreement with QFT-GIT (95.2%, κ=0.91) [24]. Data from people with active TB are lacking. The tests are CE marked and available in EU and other countries.

STANDARD F TB-Feron (IFN-gamma) uses three tubes containing recombinant protein antigens (ESAT-6, CFP-10 and TB7.7). The test requires a STANDARD F2400 analyser and returns quantitative values, which can be interpreted in the same way as QFT-GIT. Unlike ichroma II and QIAreach, the analyser is not battery operated. The test is already CE marked and is available in the EU. Published data on its performance are lacking.

AdvanSure I3 TB-IGRA is a chemiluminescent assay designed to use an automated analyser, AdvanSure I3, to quantify IFN-γ response to three *M. tuberculosis*-specific antigens (CFP-10, ESAT-6 and TB 7.7). Similar to IGRAs using fluorescence lateral flow assays, this test is easier to use and has a faster turnaround time (15 min post-incubation) than ELISA-based tests. A study in the Republic of Korea using 341 blood samples from healthcare workers and patients screened for latent TB infection or active TB demonstrated excellent agreement between AdvanSure I3 TB-IGRA and QFT-GIT (99.1%, κ=0.98) [25].

VIDAS TB-IGRA (bioMérieux, Marcy l'Etoile, France) is a fully automated solution performed on the VIDAS3 instrument. The test uses an enzyme-linked fluorescent assay to measure IFN-γ after an automated *in vitro* stimulation with ESAT-6 and CFP-10 peptide antigens together with an enhancer of cellular immunity. The blood sample can be collected in a single heparin tube. The sample and stimulants are distributed by the automated pipetting unit of the VIDAS3 in three different strips, followed by 16 h incubation in the instrument and analysis. It takes 17 h from sample loading to results and four samples can be tested per run. VIDAS TB-IGRA is not yet commercially available, whereas the VIDAS3 instrument is. The manufacturer plans to launch the test in the EU in 2021 and in the USA in 2022. A validation study is currently ongoing (ClinicalTrials.gov: NCT04048018).

Novel in vitro *tests for TB infection*

While all of the *in vitro* tests described so far employ IFN-γ as a readout marker, alternative markers have been explored to increase the diagnostic performance of IGRA. IFN-γ-induced protein 10 (IP-10) has been most extensively investigated [26]. IP-10 is a chemokine secreted by antigen-presenting cells upon stimulation by multiple cytokines including IFN-γ. Compared with IFN-γ, its expression is reported to be 100-fold higher [26]; hence the use of IP-10 as a readout marker is speculated to increase analytical

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TABLE 3 Characteristics of *in vitro* tests for tuberculosis (TB) infection, whole-blood interferon (IFN)-γ release assay (IGRA) with lateral flow assays (LFAs) or other types

	AdvanSure I3 TB-IGRA	Erythra TB test	GBTsol Latent TB Test Kit	ichroma IGRA-TB	IP-10 IGRA LFA	LIAISON QuantiFERON-TB Gold Plus	QIAreach QuantiFERON-TB	STANDARD F TB-Feron FIA (IFN-gamma)	VIDAS TB-IGRA
Type	WB IGRA	WB IGRA	Other	WB IGRA	WB IGRA	WB IGRA	WB IGRA	WB IGRA	WB IGRA
Antigens	EC peptides	PPD peptides	EC peptides	EC peptides	EC peptides	EC peptides	EC peptides	EC and TB7.7 protein	EC peptides
Readout	Chemiluminescence	Quantitative LFA, visual reading	No information	Quantitative LFA with reader	Quantitative LFA	Chemiluminescence	Qualitative fluorescent LFA with reader	Quantitative LFA with reader	Enzyme-linked fluorescence assay
Marker	IFN-γ	No information	No information	IFN-γ	IP-10	IFN-γ	IFN-γ	IFN-γ	IFN-γ
Interval before sample processing	No information	Within 6 h (18–25°C) or 32 h (2–8°C)	Within 24 h	Within 16 h (2 h recommended)	No information	Within 16 h or 48 h at 2–8°C if drawn into heparin tubes	Within 16 h or 48 h at 2–8°C	Within 16 h	Within 6 h (18–25°C) or 32 h (2–8°C)
Incubation time	37 °C, 16–24 h	Not required	1 h	16–24 h	16–24 h	16–24 h	16–24 h at 37°C without CO_2	16–24 h	Integrated as part of automation
Assay time[#]	15 min	20 min	1 h	15 min	16 h	46 min	20 min	15 min	17 h[¶]
Throughput	2 samples per run	1 sample per run	20 tests per kit	ichroma II: single test per run; ichroma 50: up to 60 tests per hour	1 test per run	Up to 25 samples per hour	8 samples per run	1 test per run	4 samples per run
Regulatory approval	No information	To be determined	Planned at end of 2021	CE	No information	CE and FDA	Planned in quarter 1 of 2021	CE	Planned in 2021 (CE) and 2022 (FDA)

WB: whole blood; EC: early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10); PPD: purified protein derivative; IP-10: IFN-γ-induced protein 10; CE: European conformity mark; FDA: Food and Drug Administration. [#]: time from post-incubation to results; [¶]: incubation is integrated.

accuracy. Currently, two whole-blood IGRAs using IP-10 are in the pipeline, based on an ELISA or a lateral flow assay. Both of them are being developed by R-Biopharm (Pfungstadt, Germany) and use CFP-10 and ESAT-6 peptide antigens for stimulation. Limited information is available since the manufacturer did not participate in the survey.

Other novel tests are in the pipeline. The GBTsol Latent TB Test Kit (Glory Biotechnologies, Seoul, Republic of Korea) is based on a novel technology based on direct detection of antigen-specific T-cells through binding of major histocompatibility complex (MHC)-II with ESAT-6 peptides and MHC-I with CFP-10 peptides, to the T-cell receptor of ESAT-6 and CFP-10 specific CD4 and CD8 T-cells, respectively. The MHC–peptide complexes will be conjugated with biotin for fluorescence detection with patented technology for micro-filter separation of whole-blood cells. In contrast to other *in vitro* tests for TB infection, the GBTsol Latent TB Test Kit requires only 1 h to return results including incubation. The Erythra TB test (Erythra, Stanford, CA, USA) is a lateral flow chromatography assay, but information is limited to validate its performance. More data on the performance of these novel technologies are awaited.

M. tuberculosis-*specific skin tests*

Several skin tests using *M. tuberculosis*-specific antigens are available. We identified five such tests, four of which use *M. tuberculosis* complex-specific ESAT-6 and CFP-10 antigens: Diaskintest (JSC Generium, Moscow, Russian Federation), EC-Test (recently renamed as C-TST) (Anhui Zhifei Longcom Biopharmaceutical, Anhui, China), C-Tb (Serum Institute of India, Pune, India) and Identification Allergen (Zhejiang Hisun Pharmaceutical, Tai Zhou, China). The fifth test uses DPPD antigen (HDT Bio, Seattle, WA, USA). In all of these tests, like the TST, skin reactions need to be read 48–72 h after intradermal injection (table 4).

Diaskintest and C-TST are commercially available. Diaskintest has been widely available in Russia and its neighbouring countries since 2008, while C-TST is available in China. Both contain a recombinant fusion protein of ESAT-6 and CFP-10, and appear to have similar accuracy to existing IGRAs. In a study among participants with suspected pulmonary TB, Diaskintest and QFT-GIT were concordant in 84% of adults and 90% of children, respectively (κ=0.63 and 0.80, respectively) [27]. In a small number of adults with bacteriologically or histologically confirmed TB (n=17) in the same study, the sensitivity of Diaskintest and QFT-GIT was 71% and 82%, respectively [27]. According to the results from a phase 3 study described in the package insert of C-TST, the test had a comparable sensitivity to T-SPOT.TB and TST (C-TST 90.6%; T-SPOT.TB 91.1%; TST 90.9%) in patients with active TB. The specificity of C-TST evaluated in healthy individuals was also similar to T-SPOT.TB (88.2% *versus* 93.2%).

The C-Tb skin test contains a mix of recombinant ESAT-6 (dimer) and CFP-10 proteins, and its performance has been rigorously evaluated in multiple countries and various populations including PLHIV and children. In a phase 3 study, C-Tb results were highly concordant with QFT-GIT in healthy volunteers, occasional TB contacts and close TB contacts (94%, κ=0.83), although its sensitivity in active TB patients was lower than QFT-GIT (67% *versus* 81%) [28]. In the same study, C-Tb positivity was highly correlated with the degree of exposure to TB. Furthermore, C-Tb was shown to be less affected by CD4 T-cell counts than the TST and IGRAs, and thus it can be used with a universal cut-off of 5 mm [29]. The planned date for market launch is yet not known.

TABLE 4 Characteristics of specific skin tests

	C-Tb	Diaskintest	DPPD	C-TST	Identification Allergen
Antigen	rdESAT-6 and CFP-10	ESAT-6/CFP-10 fusion protein	rv0061	ESAT-6/CFP-10 fusion protein	ESAT-6/CFP-10 fusion protein
Positive reaction	Induration ⩾5 mm	Infiltrate of any size	Induration ⩾10 mm or ⩾5 mm in PLHIV	Induration ⩾5 mm	Induration ⩾5 mm
Storage	2–8°C	2–8°C; storage for up to 7 days at <25°C	Unknown	2–8°C	Unknown
Regulatory approval	In process	Russia, Belarus, Kazakhstan, Moldova, Azerbaijan, Uzbekistan	No	National medical product administrations (China)	No information

rd: recombinant dimer; ESAT-6: early secreted antigenic target 6; CFP-10: culture filtrate protein 10; PLHIV: people living with HIV

Little information is available for the other two tests, as the manufacturers did not participate in the survey. Identification Allergen is produced by a Chinese manufacturer, and it contains a fusion protein of ESAT-6 and CFP-10 [30]. The DPPD-based skin test contains a recombinant protein rv0061, named DPPD. The gene coding DPPD is present only in the *M. tuberculosis* complex (including *Mycobacterium bovis*-BCG) and is absent in NTMs [31]. Thus, this test may be a more specific alternative to the TST in settings without BCG vaccination. More data are needed to evaluate its utility.

Needs and priorities

Our survey identified a number of new tests for TB infection. They include IGRAs using a simple assay like lateral flow, which are expected to facilitate decentralising tests for TB infection in peripheral facilities. New skin tests will likely increase access to more specific tests than the TST at the community level. However, several gaps exist.

First, data from well-designed studies that are sufficient to inform WHO policy are limited. For example, while a number of publications on Diaskintest are available, mostly in Russian journals or as conference abstracts, the studies were commonly conducted by retrospective analysis using data from routine settings. Hence, they were not designed to study the performance of tests. Therefore, these studies tend to suffer from incorporation bias by inclusion of people diagnosed with active TB based on the TST or Diaskintest itself as well as insufficient reporting. Very few studies are available for other tests, which, when available, were conducted in limited settings. Data among various populations such as PLHIV and children are scarce. Until now, among tests not yet endorsed by the WHO, QFT-Plus and C-Tb have been the most rigorously and extensively evaluated. The WHO recently published a framework that provides guidance on evaluating the performance of tests for TB infection using a standardised study design [32]. Manufacturers are encouraged to adopt the standard design, and funders and other stakeholders should promote it to expedite the introduction of new tools into WHO policy. Furthermore, sharing of data should be encouraged to enable rigorous head-to-head evaluation of different tests through individual patient data meta-analysis, which can better inform policy development than aggregated data meta-analysis.

Second, most *in vitro* tests for TB infection are based on the same technological concept as the existing IGRAs and thus have inherent limitations. These tests require incubation for 16–24 h, precluding same-day diagnosis. Because of the need for viable cells, blood samples must be processed within 16 h after sample collection or at a maximum of 48 h if drawn into heparin tubes and stored under refrigeration. This requires availability of tests in all peripheral facilities where samples are collected or a strong network enabling frequent transportation of samples. For T-SPOT.TB, the use of an optional test kit allows sample storage at room temperature for up to 54 h. Similar innovations should be explored to allow flexibility in sample storage and transportation. Moreover, IGRAs require phlebotomy, which is challenging for children and is not necessarily possible by lay health workers. A novel test like the GBTsol Latent TB Test Kit may overcome some of these challenges but it is still at an early stage of development. Also, it is not possible to determine the drug-susceptibility profile of infected strains as these tests only measure immune response. In addition, the use of a different, more sensitive readout as in the case of QFT may require a re-evaluation of the cut-off and the grey zone.

Similarly, skin tests are associated with the same operational challenges as the TST. Training is necessary for standardised administration of skin tests and reading of results. A need for manual operation makes quality control challenging. A return visit is necessary for reading results. These skin tests require a 2–8°C cold-chain for storage and transportation. New technologies may overcome logistic barriers associated with administration and reading of skin tests. The use of a micro-needle patch or a jet injector could enable untrained healthcare workers to administer skin tests in a standardised manner [33, 34]. Researchers developed software to measure skin induration size of the TST using a smartphone camera, which showed an excellent agreement with standard readers (intraclass correlation coefficient 0.97) [35]. This can remove a return visit and if combined with a micro-needle patch, even self-testing and reading might be possible. Research on such innovative tools that can facilitate implementation of skin tests should be promoted and studies combining new skin tests with these technologies are awaited.

Lastly, none of the new tests in the pipeline were evaluated in cohort studies and thus no data exist on their predictive performance for future development of active TB. Therefore, it was not possible to compare their performance against targets defined by the WHO [9]. Nevertheless, since most of them use the same antigens as the existing tests, *i.e.* ESAT-6 and CFP-10, with or without some modification, it is unlikely that these tests offer significant improvement in predictive performance. We need a test that can more accurately predict development of active TB so that we can expand TPT beyond high-risk groups and accelerate reduction of TB incidence and deaths.

Looking forward: tests for incipient TB

Current tests for TB infection do not differentiate individuals in the various stages from infection to active TB. These tests measure immune sensitisation by *M. tuberculosis*, *i.e.* evidence of exposure; hence, a test remains positive even after clearance of TB bacilli. A test for incipient or subclinical TB [9] is needed to accurately predict likely development of active TB in the near future. Such tests could also help find subclinical TB, which accounts for 50% of active TB found in prevalence surveys [36].

Among various approaches proposed to identify incipient TB and achieve better prediction of TB development, the use of the mRNA signature has been extensively studied and successful. Unlike IGRAs, which require stimulation of lymphocytes and hence incubation, it can characterise the host response to TB in unstimulated blood [37]. Systematic reviews identified at least 25 mRNA signatures [38–40]. In an individual participant data meta-analysis, eight out of 17 signatures had equivalent accuracy for prediction of progression to active TB over 2 years based on areas under the receiver operating characteristic curves ranging from 0.70 to 0.77 [40]. Although these signatures did not achieve the minimum target for predictive performance set by the WHO (⩾75% sensitivity and ⩾75% specificity) [9], they achieved it over a short time frame (0–3 months) [40]. While tests for incipient TB were not within the scope of our landscape analysis, we identified a few tests for incipient TB under development. QuantuMDx (Newcastle upon Tyne, UK) is developing a point-of-care test using correlate-of-risk six-gene signatures. The test can be done with finger-prick blood, returns results in 1 h and is battery operable, making it a suitable test for use at the community level. Cepheid (Sunnyvale, CA, USA) developed an early prototype GeneXpert PCR test that can measure a three-gene host response mRNA signature using whole-blood samples. Its first evaluation study was conducted to evaluate its performance as a triage test or a confirmatory test for active TB in PLHIV, rather than a test for progression in otherwise healthy individuals [41]. Yet, the same three-gene signature was identified as one of the best-performing signatures for prediction in the aforementioned review [40], using *in silico* validation of published datasets. Thus, the same platform could be used as a test to predict development of active TB. bioMérieux is developing a 30-marker transcriptomic assay for the BioFire platform, although no data are publicly available yet. Proteomic signatures for incipient TB have also been developed and validated [42]. While these tests using transcriptomic or proteomic signatures are likely to have better predictive performance than the current tests for TB infection, the value of these tests to identify targets for TPT needs evaluation. The CORTIS trial did not find any reduction of TB incidence when 3-month weekly rifapentine and isoniazid was given based on results of an 11-gene transcriptomic signature of TB risk [43].

Conclusions

We have summarised the latest landscape of tests for TB infection. Promising new tests may bring diagnosis for TB infection and prognosis of TB disease closer to the people who are in need. Rapid access to these tests would need to be ensured once endorsed by the WHO. More investment is needed in research and development of tests to allow rapid, accurate and easy identification of populations who would benefit the most from treatment. The COVID-19 pandemic has reminded us of the power of global commitment and solidarity, which dramatically accelerated research and development of diagnostics, vaccines, treatment and infrastructure for COVID-19. At the United Nations High Level Meeting on TB, global leaders committed to increasing funding for TB research and development to USD 2 billion annually. However, the funding figure in 2018 was less than half the annual target and that for diagnostics was reduced from the previous year [44]. Lessons learned from the COVID-19 pandemic should lead to adequate and equitable funding for research on TB, the single greatest cause of mortality due to an infectious disease that has been a global emergency since 1993.

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