EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Review

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Please cite this article as: Hamada Y, Cirillo DM, Matteelli A, *et al*. Tests for tuberculosis infection: landscape analysis. *Eur Respir J* 2021; in press (https://doi.org/10.1183/13993003.00167-2021).

TESTS FOR TUBERCULOSIS INFECTION: LANDSCAPE ANALYSIS

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A 256-character (including spaces) summary: New tests for tuberculosis infection are emerging that have the potential to improve accuracy, operational characteristics and end-user access. Evaluation of these tests in a standardized design would facilitate their endorsement and programmatic scale-up.

ABSTRACT

Only tuberculin skin tests (TST) and two interferon-γ release assays (IGRA) - 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 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.

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

We identified 20 tests for TB infection including 15 *in-vitro* tests and five skin tests. Thirteen of the in-vitro tests are whole-blood IGRA and 14 uses early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), with or without additional antigens. Ten are based on assays other than an enzyme-linked immunosorbent assay such as a fluorescent lateral flow assay, which requires less manual operation and shorter assay time and hence is more suitable for decentralization compared to the existing IGRA.

Four of the five skin tests use ESAT-6 and CFP-10 proteins while the remaining one uses a new antigen that is specific to *Mycobacterium tuberculosis* complex.

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 standardized 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, Member States committed to provide TB preventive treatment 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 contact.[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 TB preventive treatment.[4]

The uptake of TB preventive treatment 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 TB preventive treatment.[5, 6] Programmatic implementation of current tests for TB infection is fraught with difficulties. Manufacturing challenges in tuberculin skin tests (TST) have led to periodic shortages[7] and access is hampered by the requirement to maintain cold-chain for transportation and storage. High cost and inadequate laboratory infrastructure make it difficult to implement the alternate test for infection, interferon-γ release assays (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, that measures immune response to stimulation by *Mycobacterium tuberculosis* (MTB) antigens, 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 TB preventive treatment in people living with HIV and household contacts under five 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 people living with HIV 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 health care. Rapid tests (e.g. less than one hour 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 article is to summarize tests for TB infection in the market and in the pipeline and highlight gaps and priorities.

Tests for new TB infection were identified through an online survey targeting test manufacturers (Box 1).

Box 1. Online survey to identify tests for tuberculosis infection

We conducted a web-based survey targeting diagnostic 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 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 the test, readout, antigens), operational characteristics, the 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 *Mycobacterium tuberculosis* antigens and are intended for identifying individuals to be given treatment for TB infection were included in the review.

Thirteen manufacturers participated in the survey providing information on 14 tests for TB infection (11 *in-vitro* tests and 3 skin tests) and one test, which 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.

Table 1. List of all manufacturers and tests identified

Survey response	Name of company	Country	Name of test
Yes	bioMérieux	France	VIDAS TB-IGRA
Yes	Boditech Med Inc.	Republic of Korea	ichroma IGRA-TB
Yes	Erythra Inc.	United States	Erythra TB test
Yes	Glory Biotechnologies Corp.	Republic of Korea	GBTsol Latent TB Test Kit
No	LG Chem	Republic of Korea	Advansure I3 TB-IGRA Advansure TB IGRA

In-vitro test	Yes	LIONEX Diagnostics & Therapeutics GmbH	Germany	LIOFERON TB/LTBI
	Yes	Oxford Immunotec	United Kingdom	T-SPOT.TB
	Yes	QIAGEN	The Netherlands	QuantiFERON-TB Gold Plus QIAreach QuantiFERON-TB Gold Plus LIAISON QuantiFERON-TB Gold Plus
	Yes	QuantuMDx	United Kingdom	Unspecified (Correlate of Risk*)
	Yes	rBiopharm	Germany	IP-10 IGRA LF 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 Co., Ltd	China	EC-Test
	Yes	JSC Generium	Russian Federation	Diaskintest
	Yes	Serum Institut of India	India	C-Tb
	No	Zhejiang Hisun Pharmaceutical Co., Ltd	China	Identification Allergen
	No	Infectious Disease Research Institute	United States	DPPD

*This test was deemed a test for incipient TB

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

Tests for TB Infection Currently Recommended by WHO

Tuberculin skin test (TST) also known as Mantoux test uses purified protein derivative (PPD), a mixture of antigens obtained from MTB. Intradermal injection of PPD induces a delayed-type hypersensitivity reaction and the diameter of the induration is measured in millimetres 48-72 hours after injection. TST is affected by cross-reactions with bacille Calmette-Guerin (BCG) vaccine and non-tubercular mycobacteria (NTM) as PPD contains proteins found in most mycobacterial species.[11] The impact of BCG on 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 TST 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 such as those taking immunosuppressive agents and PLHIV.[13] Because of these multiple factors affecting TST reaction, the cut-off usually varies depending, for example, on history of BCG vaccination, prevalence of NTMs, and presence of conditions impairing immunity.[13]

Advantages of TST include not requiring laboratory infrastructure or technicians and its low cost. Unlike IGRA, phlebotomy is not necessary. On the other hand, administration of TST and interpretation of skin induration requires training and standardizing administration and reading and ensuring their quality is a challenge. The need for a return visit to read results increases barriers to patients. Cold-chain is required for transportation and storage of PPD. Several PPD products are available, of which PPD-S2 (Aplisol® (JHP Pharmaceuticals, Inc, Rochester, USA) and Tubersol® (Sanofi Pasteur Limited, Swiftwater, USA) and PPD RT23 (AJ Vaccines, Denmark) are used widely.[11] The potency of the standard dose of PPD RT23 and PPD-S2 is considered equivalent; however, PPD standardized 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 interferon (IFN)-γ release following stimulation by antigens specific to MTB. In 2011, WHO reviewed the evidence on the performance of two types of IGRA, QuantiFERON-TB Gold In-Tube (QFT-GIT) (QIAGEN, the Netherlands) and T-SPOT.TB (Oxford Immunotec, UK).[14] The review did not find a significant difference in predictive performance for the development of active TB between IGRA and TST. In light of logistic challenges associated with IGRA, WHO did not recommend its use in low and middle-income countries. However, WHO updated the recommendation in 2018, recognizing global shortage of TST, and now recommends both IGRA and TST in all settings.[10] QFT-GIT is an enzyme-linked immunosorbent assay (ELISA)-based whole-blood test that uses a peptide form of antigens specific to MTB including early secreted antigenic target 6 (ESAT-6),

culture filtrate protein 10 (CFP-10), encoded by 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 the 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 people living with HIV 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, immigrant, health care workers, and immunocompromised patients.[15] A recently published study reported that HIV status or CD4 cell count did not significantly affect IFN-γ level due to retention of 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 two years and the predictive performance was similar to that reported in TSPOT.TB and QFT-GIT.[17]

T-SPOT.TB (Oxford Immunotec, UK) measures the number of peripheral mononuclear cells that produce IFN-γ in response to ESAT-6 and CFP-10 by the enzyme-linked immunospot (ELISPOT) assay. The laboratory procedure is more complex for TSPOT.TB than QFT. The use of T-cell Xtend reagents enables isolation of lymphocytes for up to 32 hours (in contrast to 8 hours 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 hours 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 TST. However, because these antigens are not present in most NTMs (thereby excluding detection of sensitization to BCG strains and those

NTMs other than *M. marinum, M. kansasii, M. szulgai, M. flavescens*s), 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 summarized below.

There are 13 *in-vitro tests* for TB infection in the pipeline, 12 of which are whole-blood IGRA and one, 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

Table 2 and 3 summarize characteristics of *in-vitro* tests for TB infection.

Table 2. Characteristics of existing and new ELISA or ELISPLOT-based IGRAs

	Advansure TB	IP-10 Elisa	LIOFERON TB/LTBI	QFT-Plus	Standard E TB Feron	TSPOT.TB
Type	WB IGRA	WB IGRA	WB IGRA	WB IGRA	WB IGRA	PBMC IGRA
Antigens	EC peptides	EC peptides	EC, TB7.7, alanine dehydrogenase 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	In a single heparin tube and distribute into 4 tubes	In 4 specialized tubes or a heparin tube and distribute	In 3 tubes specialized tubes or a heparin tube and distribute	Single heparin or a specialized tube (Vacutainer CPT)
Interval before sample processing	No information	No information	Within 16 hours	Within 16 hours or 48 hours at 2°C - 8°C.if drawn into heparin tubes	Within 16 hours	Within 8 hours, 32 hours with T Cell Xtend, or 54 hours with T Cell Select
Incubation time	16-24 hours	16-24 hours	16-24 hours	16-24 hours	16-24 hours	16-24 hours
Assay time[1]	2 hours	20 minutes	2.5 hours	3 hours	1.5hours	4 hours
Regulatory approval	CE	CE	CE	CE, US-FDA	CE	CE, US-FDA

ELISA: Enzyme-Linked Immunosorbent Assay; ELISpot: enzyme-linked immunosorbent spot; WB: Whole blood; PBMC: Peripheral Blood Mononuclear Cells; EC: ESAT-6 and CFP-10; IFN-γ: Interferon-γ; US-FDA: U.S. Food and Drug Administration

[1] Time from post-incubation to results

Table 3. Characteristics of *in-vitro* tests for TB infection, whole blood IGRA with lateral flow assays or other types.

	Advansure i3 TB-IGRA	Erythra TB test	GBTsol Latent TB Test Kit	ichroma IGRA-TB	IP-10 IGRA LF	LIAISON QuantiFERON-TB Gold Plus	QIAreach QuantiFERON-TB	STANDARD F TB-Feron FIA	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 TB 7.7. protein	EC peptides
Readout	Chemo-luminescence	Quantitative LFA, visual reading	No information	Quantitative LFA with reader	Quantitative LFA	Chemo-luminescence	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 hours (+18°C/+25°C) or 32 hours (+2°C/+8°C)	Within 24 hours	Within 16 hours, (2 hours recommended).	No information	Within 16 hours or 48 hours at 2°C to 8°C if drawn into heparin tubes	Within 16 hours or 48 hours at 2°C to 8°C.	Within 16 hours	Within 6 hours (+18°C/+25°C) or 32 hours (+2°C/+8°C)
Incubation time	37°C, 16–24 hours	Not required	1 hour	16-24 hours	16-24 hours	16-24 hours	16-24 hours at 37 °C without CO2	16-24 hours at	Integrated as part of automation
Assay time[1]	15 min	20 minutes	1 hour	15 minutes	16h	46 min	20 minutes	15 minutes	17 hours[2]
Throughput	2 samples per run	1 sample per run	20 test 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 in end of 2021	CE	No information	CE and US-FDA	Planned in Q1 2021	CE	Planned in 2021 (CE) 2022 (US-FDA)

LFA: Lateral flow assay; WB: Whole blood; EC: ESAT-6 and CFP-10; PPD: Purified protein derivative; IFN-γ: Interferon-γ; US-FDA: U.S. Food and Drug Administration

[1]Time from post-incubation to results; [2]Incubation is integrated

Recently, QIAGEN launched LIAISON QuantiFERON-TB Gold Plus. With the LIAISON XL Analyzer (DiaSorin), 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 United States (US).

Other ELISA-based whole blood IGRAs include Standard E TB-Feron (SD Biosensor, Republic of Korea), AdvanSure TB-IGRA ELISA (LG Chem, Republic of Korea) and LIOFeron TB/LTBI (LIONEX Diagnostics & Therapeutics GmbH), 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 health care workers in a tertiary hospital in the Republic of Korea who were tested for TB infection as part of annual screening program (n=425), the concordance rate between QFT-GIT and Standard E-TB Feron was 95·3%, with the kappa value being 0·78.[22] There is no published data on its agreement with WHO-endorsed IGRA in other populations or its accuracy in people with active TB.

LIOFeron TB/LTBI uses four tubes, of which two tubes contain MTB-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 of its performance. In a

study in Italy, sensitivity in active TB patients (n=66) was 90% for LIOFeronTB/LTBI and 98% for QFT-Plus and; 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 (https://www.lgchem.com/product/PD00000230). We have little information on this test, as the company did not participate in the survey.

Simpler operation, faster results, and closer to patients

Simplified versions of IGRAs are emerging, including QIAreach QuantiFERON-TB (QIAreach) (QIAGEN, The Netherlands), "STANDARD F TB-Feron FIA (IFN-gamma)" (Standard F TB-Feron [SD Biosensor, Republic of Korea]), and ichroma IGRA-TB (Boditech Med Inc., Republic of Korea) and Advansure I3- TB IGRA (LG Chem, Republic of Korea). These tests require less manual handling than ELISA-based IGRA and the results are available in 15-20 minutes once the 16-24 hour incubation is completed. The 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 fluorescence lateral flow reader. The reader, called e-hub, is battery operable, can be connected to Laboratory Information Management Systems, and can operate for eight hours on battery supply. QIAreach is simple to use without a need for highly trained personnel; hence, it can decentralize 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 minutes. And 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 samples. This test is based on a similar technological principle to other IGRA and requires phlebotomy and incubation. In a study in 60 healthy individuals including ten TB contacts in the Republic of Korea, ichroma IGRA-TB using 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 uses three tubes containing recombinant protein antigens (ESAT-6, CFP-10, and TB7.7). The test requires a STANDARD F2400 Analyzer and returns quantitative values, which can be interpreted in the same way as QFT-GIT. Unlike ichroma-II and Access-QFT, the analyser is not battery operated. The test is already CE marked and is available in EU. Published data on its performance is lacking.

Advansure I3 TB-IGRA is a chemiluminescent assay designed to use an automated analyser, Advansure I3, to quantify IFN-γ response to three MTB 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 faster turnaround time (15 minutes post incubation) than ELISA-based tests. A study in the Republic of Korea using 341 blood samples from health care workers and patients screened for LTBI or active TB demonstrated excellence agreement between Advansure I3 TB-IGRA and QFT GIT (99·1%, kappa coefficient=0·98).[25]

VIDAS TB‑IGRA (bioMérieux, 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. 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 hours of incubation in the instrument and analysis. It takes 17 hours from sample loading to results and four samples can be tested per run. VIDAS TB‑IGRA is not yet commercially available while the VIDAS3 instrument is. The manufacturer plans to launch the test in EU in 2021 and in the US in 2022. A validation study is currently ongoing. (https://clinicaltrials.gov/ct2/show/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. Interferon-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 to 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 accuracy. Currently, two whole-blood IGRAs using IP-10 are in the pipeline, based on an ELISA and a lateral flow assay, respectively. Both of them are being developed by rBiopharm (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. GBTsol Latent TB Test Kit (Glory Biotechnologies Corp., Republic of Korea) is based on a novel technology based on direct detection of antigen-specific T cells through binding of MHC-II with ESAT6 peptides and MHC-I with

CFP10 peptides, to T-cell receptor of ESAT-6 and CFP10 specific CD4 and CD8 T cells, respectively. The MHC-peptide complexes will be conjugated with biotin for fluorescence detection with patented technology of micro-filter separation of whole blood cell. In contrast to other *in-vitro* tests for TB infection, GBTsol Latent TB Test Kit requires only one hour to return results including incubation. Erythra TB-KIT (Erythra Inc., US) is a lateral flow chromatography assay but the information is limited to validate its performance. More data on the performance of these novel technologies are awaited.

MTB-specific skin tests

Several skin tests using MTB-specific antigens are available. We identified five such tests, four of which use MTBC-specific ESAT-6 and CFP-10 antigens, including Diaskintest (JSC Generium Russian Federation), EC test (Anhui Zhifei Longcom Biopharmaceutical Co., Ltd, China), C-Tb (Serum Institute of India), and Identification Allergen (Zhejiang Hisun Pharmaceutical Co., LTD, China). The fifth test uses DPPD antigen (Infectious Disease Research Institute, US). In all of these tests, like TST, skin reactions need to be read 48-72 hours after intradermal injection (Table 4).

Table 4. Characteristics of specific skin tests

	C-Tb	Diaskintest	DPPD	EC-test	Identify Allergen
Antigen	rdESAT-6 and CFP-10	ESAT-6/CFP-10 fusion protein	rv0061	ESAT-6/CFP10 fusion protein	ESAT-6/CFP10 fusion protein
Positive reaction	Induration size>=5mm	Infiltrate of any size	Induration ≥10mm or ≥5mm in PLHIV	Induration >=5mm	Induration ≥5mm
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

Diaskintest and EC-test are commercially available. Diaskintest has been widely available in Russia and its neighbouring countries since 2008, while EC test is available in China. Both contain a recombinant fusion protein of ESAT-6 and CFP-10 and appear to have similar accuracy to existing IGRA. In a study among participants with suspected pulmonary TB, Diaskintest and QFT-GIT were concordant in 84% of adults and 90% of children, respectively (kappa values: 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 EC test, the test had a comparable sensitivity with T-SPOT.TB and TST (EC-test; 90·6%; T-SPOT.TB: 91·1%; TST: 90·9%) in patients with active TB. The specificity of the EC test evaluated in healthy individuals was also similar to T-SPOT.TB (88·2% vs 93·2%).

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 people living with HIV 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%, kappa value: 0·83) although its sensitivity in active TB patients was lower than QFT-GIT (67% vs 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 TST and IGRA 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.

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. DPPD-based skin test contains a recombinant protein rv0061, named DPPD. The gene coding DPPD is present only in the MTB complex (*including Mycobacterium bovis*-BCG) and is absent in NTMs.[30] Thus, this test may be a more specific alternative to 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 IGRA using a simple assay like lateral flow, which are expected to facilitate decentralizing tests for TB infection in peripheral facilities. New skin tests will likely increase access to more specific tests than TST at the community level. However, several gaps exist.

Firstly, data from well-designed studies that are sufficient to inform WHO policy are limited. For example, whilst a number of publications on Diaskintest are available, mostly in Russian journals or as conference abstracts, they 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 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 people living with HIV and children are scarce. Until now, among tests not yet endorsed by WHO, QFT-Plus and C-Tb have been most rigorously and extensively evaluated. WHO recently published a framework that provides guidance on evaluating the performance of tests for TB infection using a

standardized study design.[31] Manufacturers are encouraged to adopt the standard design and funders and other stakeholders should promote it to expedite 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 IGRA and thus have inherent limitations. These tests require incubation for 16-24 hours precluding the same day diagnosis. Because of the need for viable cells, blood samples must be processed within 16 hours after sample collection or at a maximum of 48 hours 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 TSPOT.TB, the use of an optional test kit allows sample storage at room temperature for up to 54 hours. Similar innovations should be explored to allow flexibility in sample storage and transportation. Moreover, IGRAs requires phlebotomy, which is challenging for children and is not necessarily possible by lay health workers. A novel test like GBTsol Latent TB Test Kit may overcome some of these challenges but it is still an early stage of development. Also, it is not possible to determine the drug-susceptible 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 to re-evaluate the cut off and the grey zone.

Similarly, skin tests are associated with the same operational challenges as TST. Training is necessary for standardized 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 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 non-trained health care workers to administer skin tests in a standardized manner.[32, 33] Researchers developed a software to measure skin induration size of TST using a smartphone camera, which showed an excellent agreement with standard readers (intraclass correlation coefficient value of 0·97).[34] 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 was 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 WHO.[9] Nevertheless, since most of them use the same antigens as the existing tests, ESAT-6 and CFP-10, with or without some modification, it is unlikely that these tests offer significant improvement in the predictive performance. We need a test that can more accurately predict development of active TB so that we can expand TB preventive treatment 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 sensitization by MTB, 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 sub-clinical TB, which accounts for 50% of active TB found in prevalence surveys.[35]

Among various approaches proposed to identify incipient TB and achieve better prediction of TB development, the use of mRNA signature has been extensively studied and successful. Unlike IGRA, which requires stimulation of lymphocytes and hence incubation, it can characterize the host response to TB in unstimulated blood.[36] Systematic reviews identified at least 25 mRNA signatures.[37–39]. In an individual participant data meta-analysis, eight out of 17 signatures had equivalent accuracy for prediction progression to active TB over two years at the areas under the receiver operating characteristic curves ranging from 0.70 to 0.77.[39] Although these signatures did not achieve the minimum target for predictive performance set by WHO ($\geq$ 75% sensitivity and $\geq$ 75% specificity),[9] they achieved it over a short time frame (0-3 months).[39] 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 is developing a point-of-care test using correlate-of-risk 6-gene signatures. The test can be done with finger-prick blood, returns results in one hour and is battery operable, making it a suitable test for use at the community level. Cepheid developed an early prototype GeneXpert PCR test that can measure 3-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.[40] Yet, the same 3-gene signature was identified as one of the best performing signatures for prediction in the aforementioned review, using *in silico* validation of published datasets. Thus, the same platform could be used as a test to predict development of active TB. Biomerieux 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.[41] 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 TB preventive treatment needs evaluation. The CORTIS trial did not find reduction of TB incidence when 3-month weekly rifapentine and isoniazid was given based on results of 11-gene transcriptomic signature of TB risk.[42]

CONCLUSIONS

We have summarized 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 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 coronavirus disease 2019 (COVID-19) pandemic 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 UNHLM on TB, global leaders committed to increasing funding for TB research and development to US$ 2 billion annually. However, the funding figure in 2018 was less than half the annual target and that for diagnostic was reduced from the previous year.[43] 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.

ACKNOWLEDGMENTS

We sincerely thank the following manufacturers that participated in our survey: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd, bioMérieux, Boditech Med Inc. Erythra Inc., Glory

Biotechnologies Corp., JSC Generium, LIONEX Diagnostics & Therapeutics GmbH, Lophius Biosciences GmbH, Oxford Immunotec, QIAGEN, QuantuMDx, SD Biosensor, and Statens Serum Institut. We also thank Oxford Immunotec and Statens Serum Institut for giving inputs to our pilot survey.

MR is an inventor on patents disclosing the use of specific skin tests and IP-10 for the diagnosis of TB infection (WO2017084671, WO2008028489, WO2012076020, WO2011137902). All rights have been assigned to Copenhagen University Hospitals and Statens Serum Institut.

Adam Penn-Nicholson is an inventor on a patent for prediction of tuberculosis disease risk (WO2017081618A9. Rights have been assigned to the University of Cape Town, and Seattle Biomed.

DMC's research team received a grant from bioMérieux, which supported a stability study for VIDAS TB-IGRA.

This work/research was supported by funding received from the Stop TB Partnership and USAID for the New Diagnostics Working Group. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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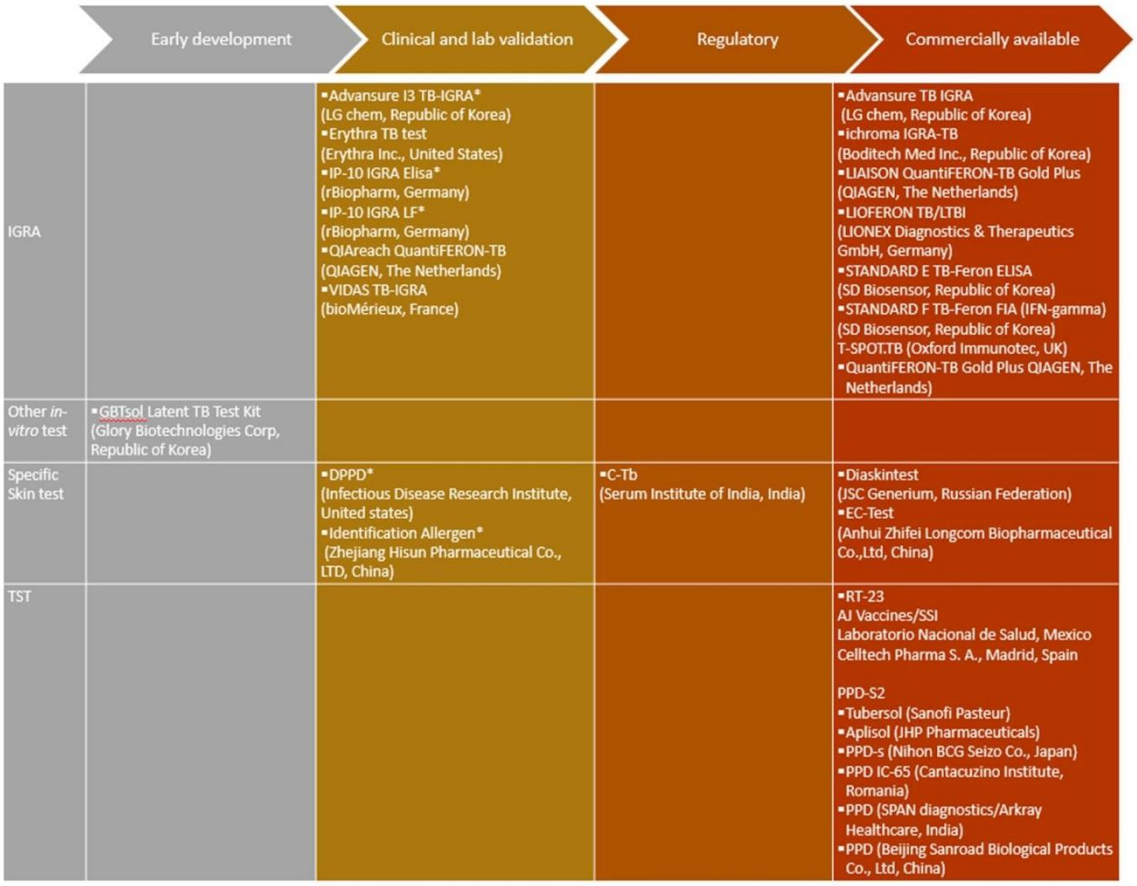
Figure legends

Figure 1. Tests for TB infection in the pipeline: at-a-glance

*Tests whose manufacturer did not participate in the survey.

Interferon-γ release assays: IGRA; tuberculin skin test: TST

Figure 1. Tests for TB infection in the pipeline: at-a-glance



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