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Use of interferon-gamma release assays in support of TB diagnosis

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Ad hoc scientific panel opinion

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Contents

Abbreviations

BCG	Bacillus Calmette-Guérin
CFP-10	Culture filtrate protein 10
CI	Confidence interval
ECDC	European Centre for Disease Prevention and Control
EU	European Union
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunosorbent spot
ESAT-6	Early secretory antigenic target-6
HAART	Highly active antiretroviral therapy
IFN-γ	Interferon-gamma
IGRA	Interferon-gamma release assay
LTBI	Latent TB infection
MS	Member State
NPV	Negative predictive value
NTM	Nontuberculous mycobacteria
NTP	National TB programme
OR	Odds ratio
PHA	Phytohemagglutinin A
PPV	Positive predictive value
QFT-GIT	QuantiFERON-TB Gold In-Tube
TB	Tuberculosis
TBNET	TB Network European Trials group
TST	Tuberculin skin test

Summary and background

This guidance document presents the evidence-based expert opinion of an ad hoc scientific panel on the use of the interferon-gamma release assays (IGRAs) for the diagnosis of latent tuberculosis (TB) infection and active TB. The panel expressed that IGRAs should not replace the existing standard diagnostic methods for the diagnosis of active TB and that a negative IGRA result does not exclude active TB disease. As to the diagnosis of LTBI, the panel expressed that IGRAs may be used in conjunction with an overall risk assessment in order to identify individuals for whom preventive treatment should be considered. Further opinions on the use of IGRAs in specific risk groups and populations are presented in the document.

IGRAs are relatively new diagnostic tools for TB. They were developed to support the diagnosis of latent TB infection but research is also ongoing for expanding its use to the diagnosis of active tuberculosis.

To assure optimal tuberculosis prevention and control it is essential that the introduction of new tools into national TB programmes and/or tuberculosis control strategie is based on solid scientific evidence. Uncertainty still remains as to the effectiveness of IGRAs. ECDC therefore identified the need to provide an EU-adapted guidance on the applicability and appropriateness of IGRAs, both for the identification of latent infection and active TB disease.

Executive summary

New tools to diagnose latent TB infection (LTBI) and active tuberculosis (TB) are needed. LTBI is commonly diagnosed by using the tuberculin skin test (TST). More recently, interferon-gamma release assays (IGRAs) were introduced for the diagnosis of LTBI.

IGRAs are blood-based tests that essentially measure the presence of specific *M. tuberculosis* reactive T-cells sensitised by a previous infection with *M. tuberculosis.* Two commercial IGRAs are available, the QuantiFERON-TB Gold In-Tube assay (QFT-GIT) (Cellestis Ltd., Australia) and the T-SPOT-*TB* (Oxford Immunotec, UK). Compared to the TST, IGRAs are not confounded by prior bacille Calmette-Guérin (BCG) vaccination and are less likely to be influenced by previous exposure to most nontuberculous mycobacteria (NTM) due to the target antigens selected to stimulate cellular immune responses.

European Union Member States are heterogeneous in terms of TB burden and characteristics of TB epidemiology, with intermediate-to-high (>20 per 100 000) and low (< 20 per 100 000) TB-incidence countries. This guidance document on the use of IGRAs in EU Member States, based on the most up-to-date scientific evidence available on the diagnosis of LTBI and active TB, was developed on the initiative of the European Centre for Disease Prevention and Control (ECDC).

The bulk of the evidence presented in this guidance document is based on two systematic reviews and meta-analyses assessing the role of IGRAs in the diagnosis of active TB and LTBI, conducted by the TB Network European trials group (TBNET) for and under the supervision of ECDC[1-2]. Additional reviews and key studies covering other areas are also presented. ECDC originally developed the document as an FAQ list (frequently asked questions) and then presented to an *ad hoc* scientific panel of experts. The panel of experts was selected by ECDC's Chief Scientist and endorsed by the ECDC Director. Experts were selected based on their expertise in different areas of TB control. It was also ensured that panel members did not have a conflict of interest. During its meeting, the panel assessed the scientific evidence on IGRAs and expressed a unanimous opinion on the use of IGRAs in defined areas of TB control.

The opinion of the panel regarding the use of IGRAs as a stand-alone test for diagnosing active TB was as follows:

Expert opinion

Based on the evidence, IGRAs should not replace the standard diagnostic methods (including microbiology, molecular tests, and clinical and radiological assessment) for diagnosing active TB.

The opinion of the panel on the use of IGRAs to *support* the diagnosis of active TB disease was as follows:

Expert opinion

Based on the evidence, IGRAs do not have an added value in most clinical situations when combined with standard methods for diagnosing active TB.

However, based on limited evidence, in certain clinical situations (e.g. patients with extrapulmonary TB, patients who test negative for acid-fast bacilli in sputum and/or negative for *M. tuberculosis* on culture, TB diagnosis in children, or in the differential diagnosis of infection with NTM) IGRAs could contribute supplementary information as part of the diagnostic work-up.

Please note that a negative IGRA does not rule out active TB.

On the question of whether IGRAs have a role in diagnosing LTBI, specifically with the aim of identifying individuals for preventive therapy, the following opinion was stated:

Expert opinion

Based on the available results on positive predictive value (PPV) for progression, and taking into consideration the low statistical power and low number of studies, IGRAs may be used as part of the overall risk assessment to identify individuals for preventive treatment (e.g. immunocompromised persons, children, close contacts, and recently-exposed individuals).

Similarly, despite the limitations of available studies, the high NPV for progression of IGRAs indicates that at the time of testing and in the context of an overall risk assessment, progression to active TB in healthy immunocompetent individuals with negative IGRAs is very unlikely. Therefore, IGRAs may be used in this context.

Please note that, especially in risk groups and specific situations, a negative IGRA does not rule out LTBI. See Section 3.2.3 'How should IGRAs be used in different population groups and settings?'.

The use of tools to diagnose LTBI must consider the accuracy of the test in specific risk groups (immunocompromised persons, children) and populations (high- or low-incidence settings, vaccination status). This document also presents the opinion of the panel on the use of IGRAs to diagnose LTBI in various populations and risk groups.

The aim of the this EU-adapted guidance on the use of IGRAS for the diagnosis of LTBI and active TB is to present the most up-to-date evidence and expert opinion regarding IGRAs, providing Member States with support when considering the introduction of IGRAs to national TB programmes and/or tuberculosis control strategies.

1 Background

1.1 Current situation

There is currently a need to identify and improve new tools for the diagnosis of latent TB infection (LTBI) and active tuberculosis (TB). LTBI is recognised as a complex clinical condition, in which the exact biological status of the TB bacilli is not fully understood[3]. When a person is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), the bacilli are thought to persist in a subclinical status with minimal replication, a status in which the bacteria are unable to cause manifest clinical disease. Upon a shift in an individual's immunologic status, *M. tuberculosis* is able to begin replicating and multiply to a number that causes disease, manifesting as active TB[3]. LTBI is therefore defined as a clinical condition in an individual suspected of being infected, but having no manifestation of disease, and from whom *M. tuberculosis* bacilli cannot be identified through culture[3].

Active TB is diagnosed by evaluating an individual's medical history, clinical symptoms, (chest) radiography, as well as the microbiologic and molecular identification of *M. tuberculosis* (through the detection of acid-fast bacilli in sputum, *M. tuberculosis* culture, and nucleic acid amplification). The diagnosis of active TB can often be challenging, with results remaining inconclusive (e.g. acid-fast bacilli sputum smear-negative), especially in specific risk groups, such as children and immunocompromised individuals[4]. New useful, sensitive, and rapid tools to detect active TB are clearly needed.

Identifying LTBI aims at identifying individuals who would benefit from treatment, preventing future development of active TB disease. This in itself is an important reason for controlling the transmission of disease within a population, as it decreases the number of active TB cases that have the potential of transmitting the infection. The challenge of identifying LTBI-infected individuals lies in the lack of a diagnostic gold standard for LTBI.

There are currently two diagnostic methods that support the diagnosis of LTBI: the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs). Both tests are immunological methods that detect an immune response to antigens and consequently do not allow a direct measure of persistent infection. The *in vivo* TST is based on the intracutaneous injection of *M. tuberculosis* antigens and subsequent identification of an immune reaction at the site of injection. A limitation of the TST is that the complex mixture of different antigens used are not specific for *M. tuberculosis*, and therefore local immunologic activity at the site of the antigen deposition does not differentiate between an existing immune response elicited by either, previous bacille Calmette-Guérin (BCG) vaccination, exposure to NTM, or *M. tuberculosis* infection[5]. IGRAs are more recent *in vitro* assays that detect the presence of cellular immune responses towards *M. tuberculosis*-specific antigens. These include the early secretory antigenic target-6 (ESAT-6), culture filtrate protein 10 (CFP-10), and the TB7.7 antigens. In contrast to the TST, the antigens in IGRAs are absent in most of NTM (with the exception of *M. flavescens*, *M. marinum, M. kansasii and M. szulgai*), as well as from BCG strains[6-8]. Although IGRAs cannot distinguish between active TB and LTBI, IGRA-results are not confounded by BCG vaccination and less likely to be confounded by exposure to NTM[9].

European Union Member States are heterogeneous in terms of TB burden and characteristics of TB epidemiology, with intermediate-to-high (>20 per 100 000), and low TB incidence countries (< 20 per 100 000). As for the application of TB diagnostic tools, a country's TB epidemiology will influence how TB control programmes consider the use of IGRAs. A number of countries have already introduced this diagnostic tool in their national TB programmes and it has been the European Centre for Disease Prevention and Control's experience that an increasing number of countries are currently considering the implementation of IGRAs and, as a result, requesting guidance and support[10-12]. In 2007, European experts developed a consensus statement on the use of IGRAs based on the scientific evidence available at the time[13]. Since then, substantially more studies have evaluated IGRAs and more countries have been considering the use of assays.

ECDC therefore identified the need to provide an EU-adapted guidance on the applicability and appropriateness of IGRAs, both for the identification of latent infection and active TB disease in order to support the Member States as they consider the introduction of IGRA's in national TB programmes and/or tuberculosis control strategies.

It should also be noted that the introduction of new diagnostic tools, including IGRAs, requires the adjustment of policies and programmes to assure that the tools are properly adopted, introduced and implemented. This is beyond the scope of this document; however, resources describing all aspects of adoption and implementation are readily available. One example is the detailed framework on adoption, introduction and implementation of new tools for TB control developed by the Stop TB Partnership Retooling Taskforce[14].

1.2 Objectives

The aim of this guidance document is to present the most recent scientific evidence and expert opinion on the use of IGRAs for the diagnosis of LTBI as well as their applicability to the diagnosis of active TB. It presents several

aspects to consider when implementing IGRAs in national TB programmes, including the accuracy of the assays, their application within different patient groups and/or TB incidence settings, and future research needs.

The ultimate goal of this document is to present the most up-to-date evidence and subsequent expert opinion regarding IGRAs, in order to provide the Member States with support when considering the introduction of IGRAs in national TB programmes and/or tuberculosis control strategies.

1.3 Document background

Following ECDC's procedures for developing evidence-based guidance, the ECDC convened an *ad hoc* scientific panel of experts in order to assess the most up-to-date scientific evidence on IGRAs, and subsequently express a unanimous opinion on the use of IGRAs in defined areas of TB control. Panel members were identified by ECDC's Chief Scientist and endorsed by the Director of ECDC, based on their expertise in different areas of TB control, as well as their documented lack of conflicts of interest (see Table 1). All members signed a Declaration of Interest, which was reviewed by the Chief Scientist who confirmed that no member of the panel had a conflict of interest in regard to the topic of discussion. During the work of the panel, one member developed a conflict of interest: the expert's status was changed to 'observer', and the expert was excluded from contributing to the conclusions of the panel.

The panel was independent from ECDC, which organised, hosted and observed the panel meeting.

Table 1: Members of the ad hoc scientific panel and observers

Name	Country	Affiliation
Markus Maeurer, Chair	Stockholm, Sweden	Karolinska Institutet and Karolinska Hospital
Gernot Rohde	Maastricht, Netherlands	Maastricht University Medical Centre
Andrew Ramsay	Geneva, Switzerland	World Health Organisation
Ibrahim Abubakar	London, United Kingdom	Health Protection Agency, Centre for Infections
Connie Erkens	The Hague, Netherlands	KNCV TB Foundation
Vera Katalinić-Janković	Zagreb, Croatia	European Reference Laboratory Network for TB (ERLN-TB)/Croatian National Institute of Public Health
Walter Haas	Berlin, Germany	Robert Koch Institute
Hans Gaines	Stockholm, Sweden	Swedish Institute for Infectious Disease Control
Anne Detjen	New York City, USA	International Union Against TB and Lung Disease
Francesco Blasi	Milan, Italy	Ospedale Maggiore (General Hospital), Milan
Christoph Lange*	Borstel, Germany	Research Centre Borstel/TBNET
Giovanni Sotgiu*	Sassari, Italy	University of Sassari/TBNET
Shreemanta Parida*	Berlin, Germany	(Former affiliation: Max Planck Institute for Infection Biology)
Elisabeth Whittaker*	London, United Kingdom	Imperial College London

** Observer*

The panel's task was to express an expert opinion on key questions referring to the accuracy of IGRAs and their applicability to national TB programmes. The evidence presented was based on systematic reviews and a meta-analyses of the literature collated by ECDC. Where such studies were not available, key studies were presented. Summaries of the results and conclusions of these studies were developed by ECDC, using a 'frequently asked questions' format, and passed on to the panel before the meeting.

The scientific evidence on the accuracy of IGRAs formed the basis for the development of this guidance document. Two systematic reviews and a meta-analyses were conducted to assess the accuracy of IGRAs in diagnosing LTBI and active TB disease. The overall aims of these reviews were to:

- evaluate the evidence on the accuracy of using IGRAs in order to diagnose active TB[1]; and
- evaluate the evidence on the accuracy/usability of IGRAs for identifying LTBI and its comparative disadvantage/advantage to the TST[2].

The objective of the first systematic review and the meta-analysis assessing IGRAs for the diagnosis of active TB was to compare the accuracy of QFT-GIT and T-SPOT.*TB* to TST in the diagnosis of active TB (in adults and in children) with blood samples, and to assess the accuracy of QFT-GIT and T-SPOT.*TB* with samples of extrasanguinous fluids in the diagnosis of active TB (in high-TB incidence and low-TB incidence settings)[1].

The objective of the systematic review and meta-analysis assessing IGRAs in the diagnosis of LTBI was to compare the accuracy (specificity, positive predictive value for progression, negative predictive value, negative predictive

value for progression, and association with *M. tuberculosis* exposure and BCG vaccination) of QFT-GIT and T-SPOT.*TB* to TST in the diagnosis of LTBI[2].

These systematic reviews were conducted by the TB Network European Trials group (TBNET) for, and under the supervision of, ECDC through an open contract finalised in March 2010. The meta-analyses on the evidence from the two systematic reviews was developed by TBNET and ECDC and published in a peer-reviewed journal, the European Respiratory Journal[1-2]. Representatives of the TBNET contractors were present as observers during the meeting to support the panel regarding the studies and results.

Also present at the meeting were two observers to support the panel in questions of TB in children and TB immunology.

Following the meeting, which was chaired by Professor Markus Maeurer, ECDC updated the document to include the panel's opinions as well as the considerations identified by the panel. The document was then sent to the panel members as well as the ECDC Advisory Forum for consultation and commenting.

1.4 Document format

The opinions of the *ad hoc* scientific panel are presented in Section 3 of this document. Preceding each opinion is a section summarising the panel's considerations, followed by an overview of the available evidence. As described above, the bulk of the evidence is based on the two TBNET/ECDC systematic reviews and the meta-analyses, which assess the accuracy of IGRAs in the diagnosis of active TB and LTBI (see Annex 1 and Annex 2)[1-2]. Where needed, the evidence has been complemented with other published meta-analyses or systematic reviews. When such studies were not available, key studies are presented.

The final section highlights future research needs and considerations regarding the use of IGRAs in the diagnosis of LTBI and active TB disease.

This document is based on the evidence and knowledge of IGRAs at the time of publication (early 2011). The current document will be updated when more evidence on IGRAs becomes available.

2 Background information on IGRAs

Upon infection with *M. tuberculosis*, the different subsets of immune cells (e.g. macrophages, T-cells) involved in the immune response directed against the bacilli do not fully eradicate the bacilli, but rather contain the infection[15]. Macrophages play an important role in the first line of defence against pathogen-infection through their ability to ingest and subsequently kill pathogens. However, having developed immune escape mechanisms, *M. tuberculosis* bacilli have the ability to persist within macrophages, averting the attack by these host cells[16-17].

The cytokine interferon-gamma (IFN-γ) is produced by different cells of the immune system: CD4 T-cells, CD8 T-cells and Natural Killer cells. This cytokine is considered to play an important role in the elimination of *M. tuberculosis* by activating the production of reactive oxygen and nitrogen intermediates in macrophages, which in turn are involved in the destruction of bacterial pathogens. T-cells specifically recognizing *M. tuberculosis* antigens, particularly CD4 T-cells[18], produce IFN-γ essential for the activation of *M. tuberculosis*-infected macrophages which, upon activation, can target *M. tuberculosis* bacilli and control their growth[19].

In summary, infection with *M. tuberculosis* triggers a complex immune response that, in most individuals, leads to the containment of the infection and the establishment of a pool of long-lasting memory T-cells specifically directed against *M. tuberculosis* antigens.

2.1 What are interferon-gamma release assays (IGRAs)?

IGRAs are blood-based tests assessing the presence of effector and memory immune responses directed against the *M. tuberculosis* antigens ESAT-6, CFP-10 and, in one of the available tests, the TB7.7 antigen. The IGRAs have been shown to predominantly measure the presence of *M. tuberculosis* -specific effector memory T-cells, the presence of which are considered indicative of previous *in vivo* exposure to the bacilli. Blood samples might also contain central-memory T-cells specific to the *M. tuberculosis* antigens and thus be measured in the assays. The latter is however seen as less likely, as this subset of cells react more slowly to antigen exposure and is considered to first release other cytokines during the time-span of the assays (e.g. interleukin-2)[3].

The IGRAs measure the presence of an adaptive immune response to *M. tuberculosis* antigens, and are thus only an indirect measure of *M. tuberculosis* exposure (evidence is still lacking as to whether an immune response corresponds to actual infection)[3, 9]. IGRAs have been developed for the identification of an immune response to *M. tuberculosis*-specific antigens, considered to be a correlate of *M. tuberculosis* infection, and are licensed for the use on blood specimens.

2.2 Which IGRAs are available?

There are two commercially available IGRAs:

QuantiFERON-TB Gold In-Tube assay (Cellestis Ltd, Australia)[20]

The QuantiFERON-TB Gold In-Tube assay (QFT-GIT), which has replaced the QuantiFERON-TB Gold assay, detects the level of IFN-γ produced in response to the *M. tuberculosis* antigens ESAT-6, CFP-10, and TB7.7, and uses the enzyme-linked immunosorbent assay (ELISA) detection method. This is an indirect measure of the presence of *M. tuberculosis* specific T-cells.

T-SPOT*.TB* assay (Oxford Immunotec, UK)[21]

The T-SPOT*.TB* measures the number of IFN-γ producing T-cells in response to the *M. tuberculosis* antigens ESAT-6 and CFP-10, and is based on the enzyme-linked immunosorbent spot (ELISPOT) assay.

Only the two commercially available IGRAs (QFT-GIT and T-SPOT.TB, both with standardised and licensed protocols that allow the comparison of study results) are considered in this guidance document.

2.3 How are IGRAs performed?

IGRAs are performed on fresh blood specimens. The QFT-GIT is performed by drawing 1 ml of blood into one of each of the three manufacturer-precoated, heparinised tubes. Within 16 hours of blood collection, the tubes must be incubated for another 16 to 24 hours at 37 °C. The plasma is harvested after centrifugation (QFT-GIT collection tubes contain a gel plug that separates the plasma from the cells when centrifuged) and used (immediately, or later, provided there is adequate storage) to assess the concentration of IFN-γ by ELISA test. Results are interpreted according to the manufacturer's recommendations (Table 1)[20].

Table 1. Interpretation criteria for the QuantiFERON-TB Gold In-Tube assay (QFT-GIT)[20]

Result	IFN-γ concentration (International Units per ml, IU/ml)		
	M. tuberculosis antigens	Nil	PHA nil
Positive	≥ 0.35 IU/ml and ≥ 25% over nil	≤ 8.0 IU/ml	Any
Negative	< 0.35 IU/ml or < 25% over nil	≤ 8.0 IU/ml	≥ 0.5 IU/ml
Indeterminate	<0.35 IU/ml or < 25% over nil	≤ 8.0 IU/ml	< 0.5 IU/ml
	Any	> 8.0 IU/ml	Any

M. tuberculosis *antigens: mixture of peptides representing the entire amino acid sequences of ESAT-6 and CFP-10, and partially TB7.7; negative control (i.e. nil), positive control (phytohemagglutinin A, PHA).*

For the T-SPOT*.TB* assay, 8 ml of blood are required and the assay must be performed within eight hours of blood collection (using, for example, heparinised tubes). Alternatively, the manufacturer also provides a reagent (T-Cell Xtend) which extends processing time to 32 hours after blood collection[21]. The T-cell-containing peripheral blood mononuclear cell (PBMC) fraction is separated from whole blood and distributed to the microtitre plate wells (250,000 cells/well) provided in the T-SPOT*.TB* assay kit. Following 16 to 20 hours (at 37°C with 5% CO2) incubation, the number of IFN-γ-secreting T-cells (represented as spot-forming units) can be detected by ELISPOT assay. Results are interpreted according to the manufacturer's recommendations (Table 2)[21].

Table 2. Interpretation criteria for the T-SPOT*.TB* assay[21]

Result	Spot count				
	M. tuberculosis antigens			Nil	PHA
	ESAT-6		CFP-10		
Positive	≥ 6 over nil	and/or	≥ 6 over nil	≤ 10	Any
Negative	≤ 5 over nil	and/or	≤ 5 over nil	≤ 10	≥ 20
Borderline*	If for any antigen highest is 5 - 7 over nil			< 10	≥ 20
Indeterminate	≤ 6 over nil	and	≤ 6 over nil	≤ 10	< 20
	Any		Any	> 10	Any

** Retesting of patient is recommended*

The presence of negative and positive controls (both in the QFT-GIT and the T-SPOT*.TB* tests) ensures that IGRAs are correctly performed. The negative control (no stimulation with the *M. tuberculosis* antigens, inducing no IFN-γ production) in IGRAs assesses the baseline level of IFN-γ present in the sample.

The positive control (PHA, a T-cell-activating mitogen) in IGRAs assesses the performance of the test by measuring the ability of T-cells to produce IFN-γ, which may be impaired in immunocompromised patients.

Indeterminate results such as a high background detected in the negative control tube, or low responses in the positive-control tube (PHA), may be explained by technical factors (e.g. inappropriate storage of blood). Indeterminate results may also be explained by the immune status of the individual being tested. For instance, individuals with an impaired immune system (e.g. low T-cells numbers, decreased capacity to respond with IFN-γ production) might show such indeterminate results.

It is recommended that new blood samples are retested when a patient's sample showed indeterminate results (or borderline results with T-SPOT*.TB* assay)[22]. If after retesting the results remain indeterminate, technical error may be ruled-out and T-cell anergy in the patient sample may be a possible explanation[23].

Please note that in the QFT-GIT assay, a standardised volume of blood, which will have a variable amount of cells depending on the sample, is tested, whereas in the T-SPOT*.TB* assay, a standardised number of cells are tested.

IGRAs were developed and licensed for use on blood. However, it is known that *M. tuberculosis*-specific T-cells are recruited at the site of infection, where their frequency is increased compared to peripheral blood. As a result, there is increased research activity on the applicability of IGRAs with extrasanguinous samples (e.g. pleural fluids, materials from bronchoalveolar lavage, ascitis, or liquor cerebrospinalis) (see Section 6.1).

2.4 What are the advantages and disadvantages of IGRAs?

As described above, IGRAs detect the presence of persistent cellular immune responses towards the *M. tuberculosis*-specific antigens ESAT-6, CFP-10 and TB7.7 (QFT-GIT), which are known to be absent in most of the NTM (except *M. flavescens*, *M. marinum, M. kansasii* and *M. szulgai*), as well as in BCG strains[6-8]. IGRAs cannot distinguish between active TB and LTBI, but results will be less likely to be confounded by an individuals' previous exposure to NTM and will not be confounded by BCG vaccination. This feature in itself shows IGRAs advantage

over the TST in identifying an individual's true immune response to *M. tuberculosis*, especially in settings/populations with high NTM exposure and general BCG vaccination.

The advantages of IGRAs over the TST are several. Most importantly, individuals being tested are only required to present once to the healthcare facility (for drawing blood), increasing the likelihood that a final diagnosis is achieved. Furthermore, these *in vitro* assays have a rapid turn-around time and, being laboratory-based, follow standardised operational procedures. This decreases the effect of inter-personal variability when conducting the assays and aids in interpreting the results.

There are a number of disadvantages with IGRAs that should be considered when introducing them to national TB programmes and/or tuberculosis control strategies: IGRA testing requires drawing blood from individuals, and drawing a sufficient amount of blood from children is difficult. Also, IGRAs have to be conducted within a limited time frame. More specifically, the blood has be tested in the laboratory within a given time-frame: 16 hours for the QFT-GIT, and eight hours for the T-SPOT.TB. The test incubation time also allows antigen-specific T-cells to react with the test antigens contained in the assay. This may trigger several immune functions, including T-cell proliferation and cytokine production, influencing the read-out of test results.

IGRAs have higher resource demands when compared to the TST, as they require laboratory access, trained personal, implementation of quality-assured procedures, and guaranteed continuous access to reagents.

As they are technically more demanding, IGRAs are a more costly diagnostic tool. However, the reading and analysis of test results can be done by batch (QFT-GIT: plasma can be frozen for later ELISA analysis), thus reducing the cost but also increasing the time until results are known. Furthermore, the higher specificity of IGRAs may decrease the number of false-positive test results when investigating LTBI, and therefore prevent further medical evaluations and treatment.

When used as diagnostic tools, two types of costs for IGRAs should be considered:

- direct costs of IGRAs; and
- overall costs, including direct and indirect costs of IGRAs.

Direct costs of IGRAs

In a cost-effectiveness analysis conducted by Pooran et al. (2010) on LTBI contact screening in the United Kingdom, IGRAs were substantially more expensive than TSTs[24]:

- QFT-GIT testing (including test kit, consumables, processing, and phlebotomy): EUR 54 (GBP 45).
- T-SPOT*.TB* testing (including test kit, consumables, processing, and phlebotomy): EUR 66 (GBP 55).
- TST testing (including disposables, administration, and reading): EUR 19.30 (GBP 16.14).

Direct and indirect costs of IGRAs

A cost analysis by Linertova et al. assessing the costs of LTBI screening in Spanish healthcare workers in 2009 factored in the time spent on testing procedures as well as the hourly wages of healthcare workers[25]. This study showed that the larger part of the cost of TST testing came from the time spent to perform the test and the reading of TST results, whereas the larger part of the cost of QuantiFERON-TB Gold (the predecessor of QFT-GIT) came from material and laboratory costs. The authors concluded that in the Spanish healthcare system the costs incurred by QuantiFERON-TB Gold and TST were similar when screening healthcare workers for LBTI.

Recent studies have assessed the cost effectiveness of IGRAs in the diagnosis of LTBI in contact screening in Germany and France, as well as healthcare worker screening in Israel[26-28]. Although QFT-GIT was shown to be more expensive than TST, QFT-GIT led to fewer false-positive results, and thus consequently to fewer chest X-ray controls, fewer prescriptions of preventive treatment (in settings of high BCG-vaccination coverage), as well as fewer clinical visits. Taking into account adherence to preventive treatment, which in the study by Diel et al.[26] was estimated to be as low as 24%, the studies concluded that QFT-GIT alone was more effective and cost-effective than TST alone.

3 Panel opinions and summary of evidence

For each question on the applicability of IGRAs, the *ad hoc* scientific panel assessed the presented evidence, identified a set of considerations, and expressed its opinion. The bulk of the evidence was based on two systematic reviews and meta-analyses conducted by TBNET for ECDC that assessed the accuracy of IGRAs in the diagnosis of active TB and LTBI (see Annex 1 and Annex 2)[1,2]. Where needed, the evidence was complemented with other published systematic reviews and meta-analyses. When such studies were not available, key studies were used.

3.1 Is there a role of IGRAs in the diagnosis of active TB?

Considerations

- Following international standards, active TB is diagnosed by evaluating a patient's medical history, conducting a physical examination, chest radiography, and identifying *M. tuberculosis* bacilli using microbiologic and molecular diagnostic methods (sputum-smear microscopy, *M. tuberculosis* culture and nucleic acid amplification)[4].
- In some instances, the clinical diagnosis of active TB is difficult and results may be inconclusive (e.g. for patients with sputum-smear, acid-fast stain negative, and/or culture-negative results), despite extensive investigation of suspected TB. New useful, sensitive, and rapid tools to detect active TB are clearly needed needed[4,29]. Such new tools for the direct detection of *M. tuberculosis* (or the corresponding genetic material) may be complemented by new indirect test methods, some of which use immunological approaches. The two latter approaches are not mutually exclusive.
- IGRAs have not been developed for the diagnosis of active TB. IGRAs identify the presence of an adaptive immune response (in peripheral blood) directed towards a defined set of *M. tuberculosis* antigens (ESAT-6, CFP-10 and, for QFT-GIT, TB7.7) and cannot differentiate between active and latent TB infection[1, 9].
- In September 2010, the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) reviewed the evidence and recommendations of an expert group on the 'Use of commercial IGRAs in low-income and middle-income countries' (typically high TB-settings and/or high HIV-burden settings), following WHO's standard procedures for policy development. The STAG-TB endorsed the 'findings of the WHO expert group and supports the strategic approach to develop negative WHO policy recommendations to discourage the use of commercial IGRAs in low-income and middle-income countries (typically high-TB settings and/or high HIV-burden settings)'[30]. This decision was based on a large body of evidence showing the poor performance of current IGRAs and the risk of increased misdiagnosis, as well as the misplacement of resources in the diagnosis of active TB in low-income and middle-income settings (typically high-TB settings and/or high HIV-burden settings)[30].

3.1.1 Can IGRAs be used as a stand-alone tool to diagnose active TB disease?

Expert opinion

Based on the evidence, IGRAs should not replace the standard diagnostic methods (including microbiology, molecular tests, and clinical and radiological assessment) for diagnosing active TB.

For the evidence on the above expert opinion, please refer to Section 3.1.2 'Can IGRAs be used to support the diagnosis of active TB disease? – Evidence'.

Upon considering the evidence, the panel identified the need to express a separate opinion on the use of IGRAs as a stand-alone tool for the diagnosis of active TB disease.

3.1.2 Can IGRAs be used to support the diagnosis of active TB disease?

Expert opinion

Based on the evidence, in most clinical situations IGRAs do not have an added value when combined with standard methods for diagnosing active TB.

However, based on limited evidence, in certain clinical situations (e.g. patients with extrapulmonary TB, patients who test negative for acid-fast bacilli in sputum and/or negative for *M. tuberculosis* on culture, TB diagnosis in children, or in the differential diagnosis of infection with NTM) IGRAs could contribute supplementary information as part of the diagnostic work-up.

Please note that a negative IGRA does not rule out active TB.

Evidence

General

The TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB included studies with specific data on sensitivity and specificity[1]. Indeterminate results were excluded before sensitivity and specificity were calculated.

Sensitivity

Sensitivity measures the ability of a test to correctly identify individuals who have a certain disease. In the context of IGRAs and the diagnosis of active TB, sensitivity denotes the proportion of individuals with known active TB who test positive when IGRAs are used, i.e. the ability of IGRAs to correctly diagnose individuals with active TB and classify them as test-positive.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB, sensitivity was assessed in patients with clinical suspicion of TB disease (*M. tuberculosis* culture-confirmed and non-confirmed cases)[1].

As listed in Table 3, the pooled sensitivity (95% CI) of QFT-GIT, T-SPOT.TB, and TST was: 80% (75-84%), 81% (78-84%) and 65% (61-68%), respectively.

Table 3. Sensitivity of IGRAs and TST in the diagnosis of active TB in patients with clinical suspicion of TB disease[1]

	Pooled sensitivity (%)	95% CI	Inconsistency I^2 (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	80*	75-84	45.3	8	348
T-SPOT.TB	81**	78-84	93.3	15	749
TST	65***	61-68	89	12	703

** Pooled sensitivity was 81% (95% CI 78-84%; I^2=0%) for patients with culture-confirmed TB.*
*** Pooled sensitivity was 92% (95% CI 90-93%; I^2=78%) for patients with culture-confirmed TB.*
**** Pooled sensitivity was 68% (95% CI 63-72%; I^2=90%) for patients with culture-confirmed TB.*

Based on the analysis, the authors of this meta-analysis concluded that the sensitivity of IGRAs was too low to support their use as rule-out tests for active TB.

Specificity

Specificity measures the ability of a test to correctly identify individuals who do not have the disease under investigation. In the context of IGRAs and the diagnosis of active TB, specificity denotes the proportion of individuals known not to have active TB and who test negative when the assay is used, i.e. the ability of IGRAs to correctly diagnose individuals who do not have active TB and classify them as test-negative.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of TB, specificity was assessed in control groups who were considered to have a low risk of being infected with *M. tuberculosis*[1]. Furthermore, studies including patients suspected of active TB (found to have an alternative disease and thus not TB) were also included in the analyses and calculations. Unlike low-risk controls, this group is more representative of patients that would be tested in a routine clinical setting for active TB.

As listed in Table 4, the pooled specificity (95% CI) of QFT-GIT, T-SPOT.TB, and TST was: 79% (75-82%), 59% (56-62%) and 75% (72-78%) respectively.

Table 4. Specificity of IGRAs and TST in the diagnosis of active TB[1]

	Pooled specificity (%)	95% CI	Inconsistency I^2 (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	79	75-82	81.1	8	569
T-SPOT.TB	59	56-62	84.5	15	1070
TST	75	72-78	89.2	12	886

As shown in Table 5, the median proportion of indeterminate results with the interquartile range (IQR) of QFT-GIT and T-SPOT.*TB* was 7% (12.6%) and 3.4% (5%), respectively.

Table 5: Median proportion of indeterminate results of IGRAs in the diagnosis of active TB[1]

	QFT-GIT	T-SPOT.TB	TST
Median proportion of indeterminate results (%)	7	3.4	n/a
Interquartile range (IQR) (%)	12.6	5	n/a

As listed in Table 6, the pooled diagnostic odds ratio (OR; 95% CI) of QFT-GIT, T-SPOT.TB, and TST was: 11.47 (5.12-25.69), 18.86 (8.72-40.77) and 5.72 (3.78-8.65), respectively.

Table 6: Pooled diagnostic odds ratio (OR) of IGRAs and TST in the diagnosis of active TB[1]

	Pooled diagnostic OR	95% CI	Inconsistency I^2 (%)	number of studies
QFT-GIT	11.47	5.12-25.69	67.8	8
T-SPOT.TB	18.86	8.72-40.77	81.2	15
TST	5.72	3.78-8.65	46.1	12

The low specificity implies that a high proportion of individuals who do not have active TB would test positive were IGRAs to be used to diagnose active TB. The studies included the assessment of IGRA accuracy not only in control groups with low risk of *M. tuberculosis* infection, but also in suspects of active TB, a group which, although free from active disease, may have LTBI. This may explain the low specificity reported for QFT-GIT and T-SPOT.*TB* (79% and 59%, respectively). The authors concluded that the low specificity of IGRAs indicated the low value of the assays in the diagnosis of active TB.

Based on the meta-analysis conducted by TBNET for ECDC that assessed the accuracy (sensitivity and specificity) of IGRAs in the diagnosis of active TB, IGRAs have a low value for diagnosing active TB, and IGRAs cannot be used as a rule-out test for active TB[1]. The authors further concluded that the low specificity of IGRAs may indicate that IGRAs are not suitable to differentiate between LTBI and active TB.

Immunocompromised persons

Immunocompromised patients (e.g. those receiving immunosuppressive drugs, patients with human immunodeficiency virus infection, HIV) represent a group that is at higher risk of reactivating a latent TB infection.

In the TBNET/ECDC systematic review and meta-analysis that assessed the accuracy of IGRAs in the diagnosis of active TB, the included studies that covered immunocompromised patients did not report stratified results[1], which precluded a specific analysis of the accuracy of IGRA tests among this population group.

A limited number of research studies assessing IGRAs in the diagnosis of active TB in immunosuppressed patients were found. Two studies assessed the accuracy of T-SPOT.*TB* (not QFT-GIT) in the diagnosis of active TB in immunosuppressed patients. Both studies were conducted in countries of higher TB incidence[31-32].

Lai et al. assessed the performance of T-SPOT.*TB* in the diagnosis of active TB in patients undergoing chronic dialysis in Taiwan[31]. As listed in Table 7, the sensitivity and specificity of T-SPOT.*TB* were 91.7% and 64.7%, respectively.

Table 7: Sensitivity and specificity of T-SPOT.*TB* in the diagnosis of active TB in patients undergoing chronic dialysis[31]

	Sensitivity (%)	Specificity (%)	Total number of subjects with determinate results
T-SPOT.TB	91.7	64.7	29

The authors concluded that these results suggest that T-SPOT.*TB* represents a sensitive tool for the diagnosis of active TB in patients undergoing chronic dialysis.

Kim et al. assessed and compared the performance of T-SPOT.*TB* in the diagnosis of extrapulmonary TB in immunocompetent and immunocompromised patients (patients with HIV, lung malignancy, liver cirrhosis, chronic renal failure, or receiving immunosuppressive treatment) in South Korea[32].

As listed in Table 8, the sensitivity and specificity (95% CI) of T-SPOT.*TB* were 96% (87-100) and 64% (51-76%), respectively, in immunocompetent patients; and 88% (68-97%) and 69% (51-83%), respectively, in immunocompromised patients.

Table 8. Sensitivity and specificity of T-SPOT.*TB* in the diagnosis of extrapulmonary TB in immunocompetent and immunocompromised patients[32]

T-SPOT.TB	Sensitivity		Specificity	
	%	95% CI	%	95% CI
Immunocompetent patients (n=113)	96	87-100	64	51-76
Immunocompromised patients (n=56)	88	68-97	69	51-83

The authors concluded that T-SPOT.*TB* had the same sensitivity in immunocompetent and immunocompromised patients (no statistical difference, p=0.32).

The number of studies addressing IGRA accuracy in the diagnosis of active TB in immunocompromised patients remains low, and in the studies presented here no conclusions on the performance of IGRAs in the diagnosis of active TB in this risk group could be drawn.

HIV-infected patients

HIV-infected individuals represent a group at higher risk of reactivating a latent TB infection. Furthermore, immunosuppression can lower the sputum bacillary load, making the diagnosis of active TB by microscopy more challenging[33]. New diagnostic tools that aid the diagnosis of active TB in this risk group are therefore urgently needed[34-35].

In the TBNET/ECDC systematic review and meta-analysis that assessed the accuracy of IGRAs in the diagnosis of active TB, the included studies did not stratify the results for immunocompetent and immunosuppressed subgroups[1]. The authors could therefore not perform an analysis of IGRA accuracy in the subgroup of patients with HIV infection.

More studies addressing IGRAs accuracy in the diagnosis of active TB in HIV-positive patients are needed to allow for the analysis of IGRAs' accuracy in this sub-group.

In a study by Clark et al. (not included in the systematic review by TBNET that assessed the accuracy of IGRAs in the diagnosis of active TB) the accuracy (sensitivity and specificity) of T-SPOT.*TB* in patients with HIV infection was determined and stratified on the basis of CD4 T-cell counts[36].

As listed in Table 9, the sensitivity for T-SPOT.*TB* in patients with <300, <200, and <100 CD4 T-cells/µl was 95.4%, 92.9% and 87.5%, respectively. Specificity was 100% for all patients groups.

Table 9: Sensitivity and specificity of T-SPOT.*TB* in the diagnosis of active TB in patients with HIV infection, stratified by CD4 T-cell count[36]

CD4 count (cells/µl)	Sensitivity (%)	Specificity (%)	Total number of subjects
<300	95.4	100	22
<200	92.9	100	14
<100	87.5	100	8

The authors concluded that T-SPOT.*TB* sensitivity was not affected by CD4 T-cell count.

Children

Children, and particularly infants and children under two years of age, exposed to active TB cases are at increased risk of establishing an infection and developing active TB (including TB meningitis). The diagnosis of TB in children is particularly challenging as symptoms can be confused with symptoms of common childhood diseases. Furthermore, sputum samples are more difficult to obtain from children, and only 10 to 15% of active TB cases in children are diagnosed by acid-fast staining of *M. tuberculosis* bacilli[37].

In the TBNET/ECDC systematic review and meta-analysis that assessed the accuracy of IGRAs in the diagnosis of active TB in children, four studies addressed the performance of IGRAs in this patient subgroup[1, 38-41].

As listed in Table 10, the mean sensitivity (SD) of QFT-GIT, T-SPOT.TB, and TST was 79.9% (20.9), 42.2% (11) and 65.4% (21.1), respectively.

Table 10: Sensitivity of IGRAs and TST in the diagnosis of active TB in children[1]

	Mean sensitivity and SD (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	79.9 (20.9)	3	491
T-SPOT.TB	42.2 (11)	3	227
TST	65.4 (21.1)	3	n/a

As listed in Table 11, the mean specificity of QFT-GIT, T-SPOT.TB, and TST was: 85.8%, 84% and 89.4% respectively (Table 11).

Table 11: Specificity of IGRAs and TST in the diagnosis of active TB in children[1]

	Mean specificity (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	85.8	1	n/a
T-SPOT.TB	84*	1	n/a
TST	89.4	2	n/a

**Note: The TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB in children misstates the mean specificity of the T.Spot-TB for one study. The correct mean specificity in this study is 84*[41]*.*

As listed in Table 12, the median proportion (IQR) of indeterminate results for QFT-GIT and T-SPOT.*TB* was 6.3% (3.6%) and 8% (2.5%), respectively.

Table 12: Median proportion of indeterminate of IGRAs in the diagnosis of active TB in children[1]

	QFT-GIT	T-SPOT.TB	TST
Median proportion of indeterminate results (%)	6.3	8	n/a
IQR (%)	3.6	2.5	n/a

In the TBNET/ECDC systematic review and meta-analysis, the analysis of IGRA accuracy in the testing of children for active TB could not be stratified by age. For instance, there was not differentiation for children under and over the age of five. Infants and children with active TB under the age of five are at increased risk for poor clinical outcome. Due to the low number of studies[1, 42-43], data for a meta-analysis of IGRAs in this vulnerable group are sparse.

The authors of the TBNET/ECDC systematic review and meta-analysis underlined the particularly low number of studies addressing the performance of IGRAs in the diagnosis of active TB in children[1]. Authors concluded that the low sensitivity and specificity of IGRAs in the diagnosis of active TB in children does not support the role of IGRAs as rule-out test for active TB.

3.2 Is there a role for IGRAs in the diagnosis of latent TB infection?

Considerations

- IGRA testing should take place in the context of an overall risk assessment for LTBI which should consider the individual's history of *M. tuberculosis* exposure, their clinical history, risk factors, chest radiography, and TST (if applicable).
- LTBI should be screened for in individuals who would benefit from preventive treatment. Screening should be conducted with the intent to determine whether preventive treatment is required.
- The clinical/biological status of LTBI varies widely. It includes individuals previously exposed and infected with *M. tuberculosis* bacilli which are in a persistent latent state (with possible undetected periods of *M. tuberculosis* reactivation/dormancy) as well as individuals previously exposed and infected with *M. tuberculosis* bacilli and with primary lesions which have become sterile over the time[3, 44].
- IGRAs do not directly measure latent infection with *M. tuberculosis* bacilli. Instead, they measure the presence of an adaptive immune response (in peripheral blood) directed towards a defined set of *M. tuberculosis* antigens (ESAT-6, CFP-10 and for QFT-GIT, the antigen TB7.7)[9].
- There is currently no gold standard for diagnosing LTBI and thus for assessing new LTBI diagnostic tools. Instead, individuals with active TB are commonly used as surrogates of LTBI to assess the accuracy of IGRAs. This represents a major limitation as the sensitivity and cut-off of IGRAs derived from individuals with active TB may not translate to individuals with LTBI[45].

- Please note that the studies reviewed in the TBNET meta-analysis and systematic review focused on low-incidence settings: the derived predictive values may differ compared with those obtained from high-incidence settings.
- Please note that the TBNET meta-analysis and systematic review included a limited number of studies and that the follow-up time of only two years for the derived negative predictive value (NPV) represents an additional limitation.

3.2.1 What is the value of IGRA tests in identifying individuals for preventive treatment?

Expert opinion

Based on the available results on positive predictive value (PPV) for progression and taking into consideration the low statistical power and the low number of studies, IGRAs may be used as part of the overall risk assessment to identify individuals for preventive treatment (e.g. immunocompromised individuals, children, close contacts, and recently-exposed individuals).

Similarly, despite the scarcity and limitations of available studies, the high NPV for progression of IGRAs indicates that, at the time of testing and in the context of an overall risk assessment, progression to active TB in healthy immunocompetent individuals with negative IGRAs is very unlikely. Therefore, IGRAs may be used in this context. However, this needs to be viewed in the context of an overall risk assessment.

Please note that a negative IGRA does not rule out LTBI. This holds particularly true in specific risk groups and specific settings. See Section 'How should IGRAs be used in different population groups and settings?'.

Evidence

Sensitivity

Sensitivity measures the ability of a test to correctly identify individuals who have a certain disease. In the context of IGRAs and the diagnosis of LTBI, sensitivity denotes the proportion of individuals with known LTBI who test positive when tested with IGRAs, i.e. the ability of IGRAs to correctly diagnose individuals with LTBI.

There is currently no gold standard for the diagnosis of LTBI and thus no method to truly confirm LTBI diagnosis. The sensitivity of IGRAs for LTBI diagnosis is therefore commonly assessed in patients with active TB, using this group as a surrogate for LTBI.

The TBNET/ECDC systematic review and meta-analysis did not assess the sensitivity of IGRAs in the diagnosis of LTBI[2].

In a meta-analysis, Menzies et al. assessed the accuracy of IGRAs in the diagnosis of LTBI. Sensitivity was determined by using patients with newly diagnosed active TB as a surrogate of latent infection[46]. This meta-analysis included studies performed in low- and high-TB-burden countries.

As listed in Table 13, the pooled sensitivity (95% CI) of QFT-GIT, T-SPOT.TB, and TST was 67% (65-78%), 87% (78-95%) and 71% (64-74%), respectively.

Table 13. Sensitivity of IGRAs and TST in the diagnosis of LTBI[46]

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
QFT-GIT	67	46-78	3	133
T-SPOT.TB	87	78-95	8	337
TST	71	65-74	14	437

The authors of this meta-analysis concluded that IGRAs have a suboptimal sensitivity for identifying LTBI. In clinical terms the measured low sensitivity would imply that a relatively high proportion of individuals with LTBI (33% for QFT-GIT and 13% for T-SPOT.TB) would test negative if tested with IGRAs.

Specificity

Specificity measures the ability of a test to correctly identify individuals who do not have the disease under investigation. In the context of IGRAs and the diagnosis of LTBI, specificity denotes the proportion of individuals known not to be infected with *M. tuberculosis* and who test negative when tested with IGRAs; i.e. the ability of IGRAs to correctly diagnose individuals who do not have LTBI and classify them as test-negative. As there is no gold standard for diagnosing LTBI, the specificity of IGRAs is commonly assessed in populations or settings with a known low or minimal risk of *M. tuberculosis* infection. This population then represents a surrogate for a group free of *M. tuberculosis* infection.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, specificity was assessed among individuals at very low risk of TB infection in low-TB-burden countries[2]. It should be noted that the TBNET analysis included only studies with defined *M. tuberculosis* cases, only covered commercially available IGRAs, and that data were stratified for low- and high-incidence countries.

As listed in Table 14, the pooled specificity (95% CI) of QFT-GIT, T-SPOT.TB, and TST was 99.4% (97.9-99.9%), 98% (86.8-99.9%) and 88.7% (84.6-92%), respectively.

Table 14: Specificity of IGRAs and TST in the diagnosis of LTBI[2]

	Pooled specificity (%)	95% CI	Inconsistency I^2 (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	99.4	97.9-99.9	0	4	346
T-SPOT.TB	98	86.8-99.9	n/a	1	40
TST	88.7	84.6-92	94.5	3	309

As listed in Table 15, the median proportion of invalid/indeterminate results, as calculated from the data of the TBNET meta-analysis and systematic review of QFT-GIT, T-SPOT*.TB* and TST was 6.43%, 11.1% and 11.1%, respectively.

Table 15: Median proportion of indeterminate results, as calculated from the data of the TBNET systematic review and meta-analysis of IGRAs and TST in the diagnosis of LTBI

	QFT-GIT	T-SPOT.TB	TST
Median proportion of invalid/ indeterminate results (%)	6.43	11.1	11.1
IQR (%)	n/a	n/a	n/a

The authors of the meta-analysis concluded that IGRAs show a higher specificity than TST in individuals with very low risk of TB infection in low-TB burden countries. The analysis indicates that IGRAs are a better LTBI diagnostic tool in these settings[2]. In clinical terms this would imply that in a low-TB-burden setting the majority of individuals not infected with *M. tuberculosis* will be correctly diagnosed as 'healthy'. The authors' conclusions were provided with the caveat that only a few studies were included in the analysis.

Positive predictive value (PPV) for progression

The positive predictive value (PPV) for progression of an LTBI diagnostic test represents the probability that an individual who tests positive is truly at risk of developing active TB disease later in life.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, PPV for progression was assessed among individuals suspected of LTBI (tested positive using IGRAs and refusing preventive treatment) and subsequently followed up for a period of up to two years[2].

As listed in Table 16, the PPV for progression (95% CI) range for QFT-GIT, T-SPOT*.TB* and TST was 2.8% (0.9-6.4%) to 14.6% (6-29%), 3.3% (1.2-7%) to 10% (1.2-32%) and 2.3 (0.7-5.2%) to 3.1% (1.4-5.8%), respectively.

Table 16: PPV for progression of IGRAs and TST[2]

	PPV for progression (%)	95% CI	Time of follow-up (months)	number of studies	N. of untreated subjects with determinate results
QFT-GIT	2.8	0.9-6.4	22 (median)	1	178
	8.3	1.8-22	19 (mean)	1	36
	14.6	6-29	24 (mean)	1	41
T-SPOT.TB	3.3	1.2-7	22 (median)	1	181
	10	1.2-32	24	1	20
TST	2.3*	0.7-5.2	24 (mean)	1	219
	3.1**	1.4-5.8	22 (median)	1	288

** TST >5mm, ** TST > 10mm*

The authors of the meta-analysis noted that there are only a few studies assessing the PPV for progression of IGRAs (only four studies were included) and that the study design varied widely, making the presented values uncertain[2]. Because of the insufficient statistical power due to the low number of studies and the small study populations, it was not possible to make a valid general statement on the PPV for progression of IGRAs. The authors highlighted the need for further research in this field.

Negative predictive value (NPV)

The negative predictive value (NPV) refers to the ability of a test to dismiss from suspicion individuals that do not actually suffer from the disease in question. It measures the probability that the patient will not have the disease when restricted to all patients who test negative. With regard to diagnosing LTBI, the NPV represents the extent to which individuals that test negative truly do not have LTBI.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, the NPV was determined by using patients with confirmed active TB, using the proportion of active TB patients with false-negative IGRA results as a surrogate for the proportion of false-negative scores in LTBI suspects (due to the lack of a gold standard for latent infection identification)[2]. The studies included in the meta-analysis were based in low-, intermediate- and high-TB burden countries.

As listed in Table 17, the pooled NPV (95% CI) of QFT-GIT and T-SPOT.*TB* was 88% (85-92%) and 94% (92-96%), respectively.

Table 17. NPV of IGRAs in the diagnosis of LTBI[2]

	Pooled NPV (%)	95% CI	Inconsistency I^2 (%)	number of studies	Total number of subjects with determinate results
QFT-GIT	88	85-92	85.1	7	362
T-SPOT.TB	94	92-96	73.3	12	739
TST	n/a	n/a	n/a	n/a	n/a

NPV for progression

The NPV for progression of an LTBI diagnostic test represents the probability that an individual who tests negative is not at risk of developing active TB later in life.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, the NPV for progression was assessed among healthy individuals in low-incidence countries that were suspected of LTBI, but subsequently tested negative[2]. The individuals were followed for an average of two years to assess whether they remained disease-free. A number of the studies included in the meta-analysis also included subjects at increased risk of developing TB disease, such as close contacts of active TB patients.

As listed in Table 18, the pooled NPV for progression (95% CI) of QFT-GIT, T-SPOT.*TB* and TST was 99.8% (99.4-100%), 97.8% (94-99%) and 99.7% (98.5-100%), respectively.

Table 18: NPV for progression of IGRAs and TST[2]

	Pooled NPV for progression (%)	95% CI	Inconsistency I^2 (%)	Time of follow-up (months)	number of studies	Total number of subjects with determinate results
QFT-GIT	99.8	99.4-100	78.1	Up to 24	4	1442
T-SPOT.TB	97.8	94-99	65.9	Up to 24	3	182
TST	99.7	98.5-100	n/a	24 (mean)	1	354

The authors of the meta-analysis concluded that the high NPV for progression measured for IGRAs indicates that an individual with a negative IGRA result will most likely not develop TB disease in the future[2]. However, the authors pointed out that the studies included in the meta-analysis only covered a small number of individuals and were restricted to follow-up periods of up to two years. Further studies on the NPV for progression would be of value.

3.2.2 Can IGRAs differentiate LTBI from active TB?

Considerations

IGRAs identify the presence of an adaptive immune response (in peripheral blood) directed towards a defined set of *M. tuberculosis antigens* (ESAT-6, CFP-10 and TB7.7)9. No evidence available at this time supports that IGRAs are able to distinguish individuals with LTBI from individuals with active TB.

Expert opinion

Based on the evidence, IGRAs are not able to differentiate LTBI from active TB. An approach relying exclusively on IGRAs should therefore not be used to differentiate LTBI from active TB.

Evidence

The authors of the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB concluded that, based on the evidence, IGRAs (which indirectly diagnose past or present *M. tuberculosis* infection) cannot differentiate LTBI from active TB[1].

3.2.3 How should IGRAs be used in different population groups and settings?

Immunocompromised persons

Considerations

- Immunocompromised individuals represent a heterogeneous group which includes patients receiving immunosuppressive treatment and patients with immunodeficiency disorders, such as chronic kidney diseases or HIV[47-48]. Impaired immune-competence can further be due to other factors, including an immature immune system (e.g. children), genetic or acquired immune defects, immunosuppression associated with other infections, malignancies or immunosuppression induced by treatment modalities (particularly treatment interfering with tumour necrosis factor-alpha (TNF-α) activity.
- Recommendations from existing guidelines or from different professional societies for the diagnosis of LTBI and active TB in immunocompromised individuals should be followed.
- It is essential to maximise the sensitivity in immunocompromised individuals in order to correctly identify as many truly infected individuals as possible.

Expert opinion

As it is essential to maximise sensitivity in immune-compromised individuals, the simultaneous use of TST and IGRAs could be beneficial in identifying LTBI. Any TST or IGRA-positive result should be taken into account in the context of an overall risk assessment when considering preventive treatment.

IGRA should thus be used as part of a comprehensive risk assessment in this group of patients in view of the high risk for TB morbidity and mortality; and prevailing national / society guidelines should be maintained and followed.

Please note that in immune-compromised individuals, IGRAs should not be used to exclude LTBI and/or active TB.

Evidence

Immunocompromised patients (e.g. patients receiving immunosuppressive drugs, or individuals with HIV) represent a group at higher risk of reactivating a latent TB infection, and screening for LTBI is therefore often recommended for this group[23]. However, only limited data are available on the accuracy of IGRAs for this high-risk group.

In immunocompromised patients, IGRA responses have been shown to be reduced compared to immunocompetent subjects, with the former group exhibiting a higher proportion of indeterminate results[46]. The presence of positive controls when running IGRAs (T-cell activation induced by PHA) allows for the assessment of test performance by measuring the ability of the sample's T-cells to produce IFN-γ, a function that may be impaired in immunocompromised patients.

Only a limited number of studies assessing the accuracy of IGRAs in the diagnosis of LTBI in immunocompromised patients were available[2]. This number was reduced even further when the inclusion criteria defined in the TBNET/ECDC systematic review and meta-analysis were applied. The systematic review therefore did not assess the accuracy of IGRAs in the diagnosis of LTBI for this subgroup.

The summaries given below were taken from a selection of reviews and studies assessing the accuracy of IGRAs in the diagnosis of LTBI in immunocompromised patients. None of these studies were selected or assessed in the TBNET/ECDC systematic review and meta-analysis. .

Richeldi et al. assessed the performance of IGRAs in the diagnosis of LTBI (Table 19) in different categories of immunocompromised patients (liver transplant candidates, HIV-infected patients, and patients with hematologic malignancies)[47].

Table 19: Results of IGRAs and TST in the diagnosis of LTBI for different categories of immunocompromised patients[47]

% of test result		Liver transplant candidates (n=120)	Patients with HIV (n=116)	Patients with hematologic malignancies (n=95)
TST	Positive	16.7*	5.2	10.5*
	Negative	83.3	94.8	89.5
QFT-GIT	Positive	26.7	3.5	26.3
	Negative	72.5	96.5	72.6
	Indeterminate	0.8	0	1.1
T-SPOT.TB	Positive	23.3	4.3	17.9
	Negative	66.7	89.7	76.8
	Indeterminate	10	6	5.3

** The percentage of positive TST results in liver transplant patients and patients with hematologic diseases was significantly different compared with the percentage of positive results of QFT-GIT and T-SPOT.TB (p<0.05).*

As listed in Table 20, the concordance (agreement of test results) between the TST and IGRAs ranged from 80.6% (TST vs T-SPOT*.TB* in liver transplant candidates) to 95.4% (TST vs QFT-GIT in patients with HIV).

Table 20: Diagnostic agreement of TST and IGRAs in the diagnosis of LTBI in different categories of immunocompromised patients[47]

Concordance (%)	Liver transplant candidates (n=108)	Patients with HIV (n=109)	Patients with hematologic malignancies (n=89)
TST vs T-SPOT*.TB*	80.6	92.7	80.9
TST vs QFT-GIT	85.2	95.4	91

Indeterminate results not included in calculations.

Richeldi et al. concluded that the performance of IGRAs in the diagnosis of LTBI varies between different categories of immunocompromised patients: in order to maximise the accuracy of LTBI diagnosis, a combined approach based on IGRAs and TST may be of value in these high-risk groups.

Segall et al. reviewed studies assessing IGRA test performance in the diagnosis of LTBI in patients undergoing chronic dialysis (LTBI was defined according to established risk factors associated with LTBI)[49].

As listed in Table 21, the sensitivity, specificity, and indeterminate results for QFT-GIT were 71.4%, 100% and 2.6%, respectively. For T-SPOT.TB, the results were 22-78.6%, 41.9-61.2% and 4.8-11% respectively.

Table 21: Sensitivity, specificity and indeterminate results of IGRAs in the diagnosis of LTBI in immunocompromised patients[49]

Patients undergoing chronic dialysis		Sensitivity		Specificity		Indeterminate results	
	Total number of subjects with determinate results	%	Number of studies	%	Number of studies	%	Number of studies
QFT-GIT	39	71.4	1	100	1	2.6	1
T-SPOT.TB	432	22-78.6	3	41.9-61.2	2	4.8-11	3

Although the number of available studies was low, Segall et al. concluded that the rate of indeterminate IGRA results for the screening of LTBI in patients undergoing chronic dialysis was also rather low.

Richeldi et al. suggested the tailored use of IGRAs for the diagnosis of LTBI in different categories of immunocompromised patients and further that caution should be taken when interpreting IGRA results in immunosuppressed patients[47]; Segall et al. concluded that – in the context of an overall risk-assessment – IGRAs should be used instead of TST in the diagnosis of LTBI in patients undergoing chronic dialysis[49].

The different conclusions drawn by the authors of the two studies described above illustrate the complexity of assessing the accuracy of IGRAs in immunocompromised patients (composed of patients with different diseases, and varying degrees of immunosuppression) in the diagnosis of LTBI. This also underlines the need for further studies assessing the accuracy of IGRAs in the different groups of immunocompromised patients.

HIV-infected patients

Considerations

- HIV/TB co-infection increases the risk of developing active TB[50]. The risk of developing active TB has been shown to double by the end of the first year of HIV-infection[51].
- It is essential to maximise the sensitivity in immunocompromised individuals in order to correctly identify as many truly infected individuals as possible.

Expert opinion

As it is essential to maximise sensitivity in immunocompromised individuals, the simultaneous use of TST and IGRAs could be beneficial in identifying LTBI. When considering preventive treatment, all positive results from TST or IGRA tests should be taken into account in the context of an overall risk assessment.

IGRA should thus be used as part of a comprehensive risk assessment in this group of individuals in view of the high risk for TB morbidity and mortality and prevailing national/society guidelines should be maintained and followed.

Please note that in immunocompromised individuals, IGRAs should not be used to exclude LTBI and/or active TB.

Evidence

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, two studies included patients with HIV infection for determining the PPV for progression[2]. The number of studies addressing the remaining variables (specificity, NPV, and NPV for progression) of IGRA accuracy in the diagnosis of LTBI TB in HIV-positive individuals was very low and no evaluation of IGRA performance in the diagnosis of LTBI in this risk group could be carried out. More studies on IGRA accuracy in the diagnosis of LTBI and active TB in patients with HIV infection are needed to allow an analysis.

As listed in Table 22, the PPV for progression (95% CI) of QFT-GIT and T-SPOT.*TB* was 8.3% (1.8-22%) and 10% (1.2-32%), respectively.

Table 22. PPV for progression of IGRAs in patients with HIV infection[2]

	PPV for progression (%)	95% CI	Time of follow-up (months)	Number of studies	Total number of subjects with determinate results
QFT-GIT	8.3	1.8-22	19 (mean)	1	36
T-SPOT.TB	10	1.2-32	24 (mean)	1	20

In order to assess individual variables on the accuracy of IGRAs in HIV-infected individuals, the guidance panel was provided with additional studies not assessed in the TBNET/ECDC systematic review and meta-analysis. These studies are presented below.

False-negative or indeterminate IGRA results, especially in patients with advanced HIV-infection and low CD4 T-cell counts, are commonly encountered in HIV-infected individuals. Preliminary studies suggest that the T-SPOT.*TB* test may be more robust as a standardised number of cells per assay is used for lower CD4 T-cell counts, whereas the QFT-GIT test uses a standardised volume of blood per assay[52-53]. This may account for the better performance of the T-SPOT.*TB* assay in patients with low CD4 counts. In a study by Richeldi et al., this trend was not observed in the HIV-positive study population[47].

Cattamanchi et al. observed a significant difference in the proportion of indeterminate results in T-SPOT.*TB* tests when comparing patients infected with HIV with different CD4 T-cell counts[54].

As listed in Table 23, the proportion of indeterminate T-SPOT.*TB* results in patients with >200, 51–200 and ≤50 CD4 T-cells/μl was 95.4%, 92.9% and 87.5%, respectively. The proportion of indeterminate T-SPOT.*TB* results were significantly different when comparing the different patients stratified by CD4 T-cell count (P=0.03).

Table 23: Proportion of indeterminate results of T-SPOT.*TB* tests in patients with HIV infection, stratified by CD4 T-cell count[54]

CD4 count (cells/ µL)	Indeterminate results (%)	Total number of subjects tested
>200	14	43
51-200	25	60
≤50	30	109

When testing HIV-infected patients with IGRAs, it is recommended that the assays be performed as early as possible in the course of the infection, before a decline in CD4 counts. Also, IGRA testing should be repeated after the initiation of highly active anti-retroviral therapy (HAART)[47].

Children

Considerations

- Children, particularly infants and children under two years of age exposed to active infectious cases, are at increased risk of infection and, if they do not receive preventive treatment, are at subsequent risk to develop active disease (including disseminated forms such as TB meningitis or miliary TB)[37]. It is therefore vital to diagnose LTBI and provide preventive treatment. In children under two years of age and/or children with immunosuppression, preventive treatment should be offered (after excluding active disease) following recent exposure in order to prevent infection and subsequent development of active disease.
- New tools to diagnose LTBI and active TB in children are urgently needed[55-56].
- It is essential to achieve the highest sensitivity of detection when diagnosing LTBI and active TB in children, particularly in children under five years of age[57].
- The diagnosis of active TB in children is particularly challenging as signs and symptoms can be confused with symptoms of other childhood diseases and clinical symptoms may be absent. Furthermore, sputum samples are more difficult to obtain from children, and only 10-15% of active TB cases in children are diagnosed by acid-fast smear-staining[37]. TB diagnosis therefore usually relies on a composite of different diagnostic tests. TST and IGRAs are sometimes added to this composite diagnosis, and positive test results indicate an increased risk for TB.
- As there is currently very little data assessing the accuracy of IGRAs in children, particularly children under five years of age, more research on the use of IGRAs, such as studies assessing the longitudinal IGRA responses in children given preventive treatment, is urgently needed.
- There is a need for a more rigorous and comparable methodological approach, including reference standards, when assessing TB diagnostic tools in children.
- When children are exposed to an infectious TB case, active TB in children under five years of age should be promptly ruled out following the diagnosis of the index case. Preventive treatment should be initiated, regardless of TST and/or IGRA results. Results should be re-evaluated after 8 to 12 weeks (with an assessment of symptoms and a TST and/or IGRA if they were initially negative) to exclude progression to active disease despite preventive treatment. In the event of a negative TST result at 8 to 12 weeks, ongoing preventive treatment should be stopped. There is no need to repeat/perform a TST or an IGRA after completion of preventive treatment in children with an initially positive test result. In children with a positive TST, but with a low risk for TB (i.e. immunocompetent child with no known exposure), a subsequent IGRA (two-step approach) may be considered to rule-out false-positive reactions caused by BCG vaccination and/or exposure to NTM.

Expert opinion

The available evidence on the use of IGRAs in children is not sufficient to change current practices and guidelines on the diagnosis and treatment of LTBI and/or active TB, particularly in children under five years of age.

Regardless of the approach chosen, these three approaches should *not* be used to rule out LTBI and/or active TB in children under five years: TST alone, IGRAs alone, a two-step approach.
If applied, IGRAs must always be performed in the context of an overall risk assessment, and decision to treat must be based on this overall risk assessment.

Evidence

In the meta-analysis by Menzies et al., assessing the accuracy of IGRAs for the diagnosis of LTBI (see Section 3.2.1 ['What is the value of IGRA tests in identifying individuals for preventive treatment?'] for full study description), the authors did not specify whether any, or which, studies included children to assess the sensitivity of IGRAs. They did, however, indicate that they could not specifically address the accuracy of IGRAs for the diagnosis of LTBI in children due to the insufficient number of studies[46].

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy (specificity, NPV, and PPV) of IGRAs in the diagnosis of LTBI, only a limited number of studies specifically addressed the use of IGRAs in children[2].

As listed in Table 24, the specificity (95% CI) for QFT-GIT, T-SPOT.*TB* and TST was 100% (91-100%), 98% (87-100%) and 55% (38-71%), respectively.

Table 24: Specificity of IGRAs and TST in the diagnosis of LTBI in children[2,58]

	Specificity %	95% CI	Number of studies	Total number of subjects with determinate results	Median age (months)	
					NTM	Other
QFT-GIT	100	91-100	1	40	44	52.5
T-SPOT.TB	98	87-100	1	40	44	52.5
TST	55	38-71	1	40	44*	52.5

Study included children with NTM and children with other forms of respiratory tract infection.
** 18/23 children with NTM and 0/22 children with other forms of respiratory tract infection had a positive TST result.*

As listed in Table 25, the NPV (95% CI) range for QFT-GIT and T-SPOT.*TB* was 95.2% (84-99%) to 97.4% (93-99%) and 92.3% (86-96%) to 95.1% (83.5-99.4%), respectively.

Table 25: NPV of IGRAs and TST in the diagnosis of LTBI in children[2]

	Range of NPV (%) (95% CI)	Number of studies	Total number of subjects with determinate results
QFT-GIT	95.2 (84-99) – 97.4 (93-99)	2	259
T-SPOT.TB	92.3 (86-96) – 95.1 (83.5-99.4)	2	255
TST	n/a	n/a	n/a

As a subgroup of children with confirmed NTM was included in the analyses, the specificity of the TST in the diagnosis of LTBI could not be determined. Furthermore, due to the limited number of studies addressing the accuracy of IGRAs in children in the diagnosis of LTBI, it was not possible to draw conclusions from the analysis.

In one of the studies (Diel et al.; included in the TBNET/ECDC systematic review and meta-analysis), the PPV for progression of QFT-GIT in contacts <16 years and ≥ 6 years old was assessed[59].

As listed in Table 26, the PPV for progression for QFT-GIT and TST was 28.6% and 15%, respectively.

Table 26: PPV for progression of QFT-GIT and TST in children <16 years[59]

	PPV for progression (%)	Number of test-positive subjects developing disease/total number with test-positive result	Follow-up time (year)
QFT-GIT	28.6	6/21	Up to 4
TST	15	6/40	Up to 4

Diel et al. concluded that the results suggest the QFT-GIT is more reliable than TST for identifying children who are at higher risk of developing active TB[59].

The authors of the TBNET/ECDC systematic review and meta-analysis underlined the particularly low number of studies addressing the performance of IGRAs in the diagnosis of LTBI in children and the urgent need for large-scale studies assessing IGRAs in this vulnerable group.

The IGRA performance in children has also been reviewed by Lewinsohn et al[43]. Indeterminate rates for IGRAs were more frequent in immunocompromised children and in young children (under five years of age), which was also observed by Tsolia et al[60]. Lewinsohn et al. concluded that children aged five years and older can be tested with IGRAs for the diagnosis of LTBI; alternatively IGRAs can be used as an adjunct to other tests for active TB diagnosis in children aged five years and older[43], accepting a positive result from either test, while combined negative results cannot exclude infection.

Altogether, the few studies addressing IGRAs accuracy in the diagnosis of LTBI in children suggest that the specificity of IGRAs is superior to that of the TST. In the diagnosis of active TB, the low sensitivity and specificity does not seem to support the role of IGRAs as rule-out test for active TB and indicate that IGRAs are not suitable to differentiate children with LTBI from children with active TB. In general, more studies in children addressing the accuracy of IGRAs in the diagnosis of LTBI and active TB are needed, particularly in children under the age of five years, who are at increased risk of poor clinical outcome upon developing active disease.

High-incidence and low-incidence TB settings/populations

Considerations

- Populations originating from high-TB incidence countries are often BCG vaccinated.
- More studies assessing the difference in IGRAs predictive value within high-TB and low-TB incidence settings are needed.
- In September 2010, the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) reviewed the evidence and recommendations of a WHO expert group on the 'Use of commercial IGRAs in low-income and middle-income countries', based on the Organization's standard procedures for policy development[30]. The STAG-TB endorsed the 'findings of the WHO expert group and supports the strategic approach to develop negative WHO policy recommendations to discourage the use of commercial IGRAs in low-income and middle-income countries (typically high-TB settings and/or high HIV-burden settings)'[30]. Regarding the use of IGRAs in LTBI diagnosis, this decision was based on a the large body of evidence discouraging the use of IGRAs to diagnose LTBI in adults, children, healthcare workers, contacts, and those involved in outbreak investigations in low-income and middle-income countries (typically high-TB settings and/or high HIV-burden settings). The STAG-TB also acknowledged the challenge to obtain high-quality data due to the lack of a reference standard to identify LTBI[30].
- As to the use of IGRAs in specific risk groups (e.g. immunocompromised persons, HIV-infected persons and/or children), see the specific sections above.

Expert opinion

In high-TB incidence countries, there is no added value in using IGRAs to diagnose LTBI, as the focus of prevention and control is to identify and treat active cases.

In low-TB incidence countries, given the evidence available, IGRAs could be used in contact tracing algorithms applying the two-step approach (following TST, in TST-positive subjects).

Evidence

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, only studies from low-TB incidence settings were included for the calculation of specificity and NPV for progression. The analysis showed that IGRAs had a higher association with exposure to *M. tuberculosis* compared with the TST[2].

As listed in Table 27, the range of OR (95% CI) of QFT-GIT, T-SPOT.*TB* and TST in the multivariate analysis studies presented in the TBNET study, in which exposure status was a predictor of test positivity, was 1.8 (0.88-3.8) to 66.8 (10.1-441), 1.2 (0.3-4.8) to 38.4 (7.59-616.11), and 0.94 (0.46-1.93) to 6.5 (1.1-36.9), respectively.

Table 27: Odds ratio (OR) of IGRAs and TST in multivariate analysis studies in which exposure status was a predictor of test positivity[2]

	Range of OR (95% CI)	Total number of subjects tested	Total number of studies
QFT-GIT	1.82 (0.88-3.8) – 66.8 (10.1-441)	1604	8
T-SPOT.TB	1.2 (0.3-4.8) – 38.4 (7.59-616.11)	434	5
TST	0.94 (0.46-1.93) – 6.5 (1.1-36.9)	1230	8

In low-TB-incidence countries, IGRAs used for the diagnosis of LTBI have also been shown not to be affected by BCG vaccination and correlated better along a gradient of exposure to *M. tuberculosis* than TST[53].

The accuracy of IGRAs in the diagnosis of LTBI in high-TB incidence settings was not the main focus of the TBNET/ECDC systematic review and meta-analysis. Separate systematic reviews and meta-analyses on such settings are needed to assess the existing evidence. In countries with high-TB incidence, exposure to NTM may be increased, including for instance exposure to the *M. leprae* homologues of *M. tuberculosis* ESAT-6 and CFP-10, which could cause positive IGRA results[61-62]. In high-TB incidence settings, the priority may be diagnosing and treating patients with active TB[53].

BCG-vaccinated and non-vaccinated individuals

Considerations

- IGRAs identify the presence of an adaptive immune response (in peripheral blood) directed towards a defined set of *M. tuberculosis* antigens (ESAT-6, CFP-10 and TB7.7) that are absent in most NTM (with the exception of *M. flavescens, M. kansasii, M. marinum* and *M. szulgai*)[9]. More importantly, these antigens are not present in any of the BCG vaccine strains, therefore eliminating the consideration of test cross-reactivity in BCG-vaccinated individuals.
- Due to different BCG vaccination policies (BCG vaccination at birth, repeated BCG-vaccination, no vaccination) in EU and non-EU countries[63], the BCG vaccination situation is not homogenous.
- It has been observed that ten years post-vaccination, BCG received in infancy has no evident effect on TST results, whereas BCG-vaccination in older age groups induces more persistent, more frequent and more pronounced TST responses[64].
- For the diagnosis of LTBI in specific settings of immunocompetent adults, please refer to Section 3.2.1.
- More studies are needed on the effect of BCG vaccination given in infancy and potential subsequent exposure to NTM, on IGRA results (short-term and long-term effect), and on the PPV of IGRAs in BCG-vaccinated people.
- Cost-effectiveness is a factor when deciding whether to use a single-test or a two-step approach (TST followed by IGRA). Logistical aspects also influence the method of choice. In a BCG-vaccinated population, a two-step approach aims at increasing test specificity (with a higher NPV).

Expert opinion

IGRAs have a clear advantage in diagnosing LTBI in BCG-vaccinated populations, as they are not influenced by BCG vaccination in terms of false-positive reactions. In a BCG-vaccinated population, IGRAs have an added value as part of an overall risk assessment, identifying individuals for whom preventive treatment should be considered.

Evidence

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of LTBI, the influence of BCG vaccination on the accuracy of IGRAs was analysed[2]. IGRAs were not affected by prior BCG vaccination and more likely to be associated with exposure to *M. tuberculosis* cases. This was assessed through multivariate analysis studies performed in low-, intermediate- and high-TB burden countries.

As listed in Table 28, the range in OR (95% CI) of QFT-GIT, T-SPOT.*TB* and TST in the multivariate analysis studies included in the TBNET review, in which BCG vaccination was a predictor of test positivity, was 0 for both QFT-GIT and T-SPOT.TB, and 3.8 (1-13.9) to 24.7 (11.7-52.5) for TST.

Table 28: Odds ratio (OR) of IGRAs and TST in multivariate analysis studies in which BCG vaccination status was a predictor of test positivity

	Range of OR (95% CI)	Total number of subjects tested	Total number of studies
QFT-GIT & T-SPOT.TB	0 (i.e. no correlation)	n/a	9
TST	3.8 (1-13.9) – 24.7 (11.7-52.5)	n/a	9

In a systematic review and meta-analysis by Pai et al. in 2008, the specificity of IGRAs in BCG-non-vaccinated and BCG-vaccinated populations was compared[65]. In respect to pooled specificity of the QFT-GIT[1], a high specificity was measured regardless of vaccination status; 99% (CI 98-100%) in BCG-non-vaccinated and 96% (CI 94-98%) in BCG-vaccinated individuals. Data are not shown here as no specific data on the QFT-GIT could be extracted from Pai et al.'s review.

As listed in Table 29, and as reported in the systematic review by Pai et al., the specificity for T-SPOT.*TB* and the pooled specificity for TST in non-BCG (or predominantly non-vaccinated) individuals is 100% and 97%, respectively. The specificity for T-SPOT.*TB* and the pooled specificity for TST in BCG-vaccinated (or predominantly vaccinated) individuals was 84.7% and 59%, respectively. As the table shows however, the values for the T-SPOT.*TB* assay are based on only one study, and the authors pointed out the limited amount of data on this assay[65].

[1] The review also included studies on the predecessor, QuantiFERON-TB Gold.

Table 29: Pooled specificity of T-SPOT.*TB* and TST for diagnosis of LTBI in BCG-vaccinated and non-vaccinated individuals[65]

	Not BCG or predominantly non-vaccinated			BCG or predominantly vaccinated		
	Pooled specificity (%)	Number of studies	Total number of subjects with determinate results	Pooled specificity (%)	Number of studies	Total number of subjects with determinate results
T-SPOT.TB	100	1	21	84.7	1	131
TST	97	6	847	59	6	551

Contact tracing

Considerations

- The aim of contact tracing is to detect LTBI in exposed individuals. Contact tracing should be conducted with the intent to provide preventive treatment.
- National guidelines for contact tracing should be followed for the most efficient and cost-effective approach.

Expert opinion

Given the available evidence, IGRAs could be used in contact tracing algorithms that use the two-step approach (following TST, in TST-positive subjects).

This combined approach is based on the need to maximise specificity while improving the cost-effectiveness of contact tracing in immunocompetent adult contacts.

Evidence

The risk for progression to active TB is at its highest during the first years following infection. Therefore, the diagnosis of *M. tuberculosis* infection in newly infected individuals who have been in contact with active TB cases is particularly important in order to provide them with appropriate preventive treatment.

The two-step approach in the diagnosis of LTBI commonly consists of a) conducting the TST, followed by b) an IGRA test. This approach is considered to improve the sensitivity and specificity of the TST results, as it may corroborate a positive TST. Conversely, it may consolidate, in certain clinical situations and risk groups, a negative TST result.

A number of expert opinions have been published regarding the use of IGRAs for contact tracing[3, 13]. Overall, most experts appear to consider a two-step approach (TST followed by IGRA) the most promising when screening contacts for LTBI.

In a 2007 workshop on the use of IGRAs in low- and medium-prevalence countries in Europe, experts agreed that applying the two-step approach for diagnosing LTBI is the optimal strategy for contract tracing. This is particularly valid in contact tracing situations in which there is a known index case (i.e. case with active TB)[13].

A TBNET consensus statement on LTBI, published in 2009, gives an overview of the different aspects to consider when conducting contact investigations for LTBI (who to screen; how to provide preventive treatment). In their statement, the experts agreed that IGRAs can be used to confirm a positive TST result in order to prevent unnecessary treatment of contacts that do not to have LTBI[3].

In 2009, a review of national guidelines on the use of IGRAs was presented at the second Global Symposium on IGRAs, giving an overview of the different strategies recommended[66]. National guidelines often recommend the use of IGRAs for diagnosing LTBI during contact tracing, with most countries favouring the two-step approach.

Lastly, the newly updated US CDC guidelines on IGRAs and the detection of *M. tuberculosis* infection state that TST or IGRAs can be used alone when conducting contact investigations[22].

Screening of occupational healthcare workers

Considerations

- The purpose of screening healthcare workers is to identify LTBI.
- Serial TSTs of BCG-vaccinated individuals can result in boosting and thus cause a false-positive result. It is therefore recommended to take into account the setting/country and its guidelines/policies on healthcare workers, as well as the healthcare workers' BCG vaccination status.
- Occupational healthcare workers are often BCG vaccinated.
- Healthcare workers may have an increased exposure to NTM in their work settings[67].
- Practices regarding the use of IGRAs in the screening of healthcare workers vary, depending on the screening objectives. Some guidelines propose IGRAs for the screening of healthcare workers that have been exceptionally exposed to TB or recommend that healthcare workers receive a screening before taking

up their jobs. However, several guidelines do not specifically mention whether IGRAs should be a preferred tool for the screening of healthcare workers[66].
- Existing national guidelines for occupational healthcare workers should be followed.
- The total exposure of healthcare workers may have an impact on the outcome or predictive value of IGRA tests.
- IGRAs may be used as a baseline test, but serial testing problems of conversion/reversion (particularly around the cut-off values for the tests) may occur[68].
- More research is needed on the use of IGRAs for the screening of occupational healthcare workers, e.g. studies assessing the accuracy of repeated IGRA testing or exploring the issue of conversion/reversion of test results.

Expert opinion

There is insufficient evidence on the PPV of IGRAs for the screening of healthcare workers to state an educated opinion on this topic.

However, given the available evidence the use of IGRAs in the two-step approach could increase the specificity depending on the population tested (e.g. BGC vaccination status).

Evidence

A review conducted by Swindells et al. concluded that IGRA testing for diagnosing LTBI in healthcare workers was beneficial but that more studies are needed (the heterogeneity of the studies assessing the role of IGRAs in the testing of healthcare workers did not allow to perform a meta-analysis)[69].

4 Future research needs and considerations

4.1 Can IGRAs be used with extrasanguinous fluids to support the diagnosis of active TB?

Considerations

- The standard diagnostic methods for active TB diagnosis are described in Section 1. However, in cases that are difficult to diagnose, all available methods should be used for the direct detection of the pathogen and its components. New microbiological and immunological diagnostic tools are needed especially for early confirmation of severe disease, for example TB meningitis.
- IGRAs were developed for blood samples and are not licensed for use with extrasanguinous fluids[20-21].
- Findings from the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB warrant continued research, particularly on the use IGRAs with extrasanguinous fluids in order to support the diagnosis of difficult-to-diagnose patients.

Expert opinion

There are certain clinical situations that urgently require supplementary tools for the direct or indirect diagnosis of active TB. Research on the use of IGRAs in extrasanguinous fluids is ongoing, but there is currently not enough evidence to support the use of IGRAs with extrasanguinous fluids in the diagnosis of active TB.

Evidence

Sensitivity

Sensitivity measures the ability of a test to correctly identify individuals who have a certain disease. When applied to IGRAs and the diagnosis of active TB, sensitivity denotes the proportion of individuals with known active TB disease who test positive when IGRAs are used; i.e. the ability of IGRAs to correctly diagnose individuals with active TB and classify them as test-positive.

In the TBNET/ECDC systematic review and meta-analysis that assessed the accuracy of IGRAs in the diagnosis of active TB, sensitivity was assessed with extrasanguinous fluids (pleural fluids, broncheoalveolar lavage or ascetic fluid) in patients with clinical suspicion of TB disease (culture-confirmed and unconfirmed TB cases)[1].

As listed in Table 30**,** the pooled sensitivity (95% CI) of QFT-GIT was 48% (39-58%); the pooled sensitivity of T-SPOT*.TB* was 88% (82-92%).

Table 30: Sensitivity of IGRAs in the diagnosis of active TB performed with extrasanguinous fluids (pleural fluids, broncheoalveolar lavage, or ascetic fluid) in patients with clinical suspicion of TB disease[1]

	Pooled sensitivity (%)	95% CI	Inconsistency I^2 (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT*	48	39-58	0	4	116
T-SPOT.TB**	88	82-92	57.9	7	186

** Pooled sensitivity was 52% (95% CI 39-64%; I^2=38%) for patients with culture-confirmed TB.*
*** Pooled sensitivity was 88% (95% CI 81-93%; I^2=22%) for patients with culture-confirmed TB.*

The authors of this meta-analysis concluded that the sensitivity of IGRAs was too low to support their role as a rule-out test for active TB.

Specificity

Specificity measures the ability of a test to correctly identify individuals who do not have the disease in question. In the context of IGRAs and the diagnosis of active TB, specificity denotes the proportion of individuals known not to have active TB disease and who test negative when IGRAs are used; i.e. the ability of IGRAs to correctly diagnose individuals who do not have active TB and classify them as test-negative.

In the TBNET/ECDC systematic review and meta-analysis assessing the accuracy of IGRAs in the diagnosis of active TB, the specificity was assessed with extrasanguinous fluids (pleural effusion, bronchoalveolar lavage fluids, or ascetic fluids) from patients with clinical suspicion of TB disease[1].

As listed in Table 31, the pooled specificity (95% CI) of QFT-GIT was 82% (70-91%); the pooled specificity of T-SPOT*.TB* was 82% (78-86%).

Table 31: Specificity of IGRAs in the diagnosis of active TB performed in extrasanguinous fluids (pleural fluids, broncheoalveolar lavage, and ascetic fluid)[1]

	Pooled specificity (%)	95% CI	Inconsistency I^2 (%)	Number of studies	Total number of subjects with determinate results
QFT-GIT	82	70-91	0	4	56
T-SPOT.TB	82	78-86	71.5	7	368

As listed in Table 32, the median proportion of indeterminate results (IQR) for QFT-GIT was 23.1% (40.1%), the median proportion of indeterminate results for T-SPOT.*TB* was 5% (8%).

Table 32: Median proportion of invalid/indeterminate of IGRAs for diagnosis of active TB performed in extrasanguinous fluids[1]

	QFT-GIT	T-SPOT.*TB*
Median proportion of invalid/ indeterminate results (%)	23.1	5
IQR (%)	40.1	9.8

As listed in Table 33, the pooled diagnostic OR (95% CI) of QFT-GIT and T-SPOT.*TB* was: 3.84 (1.73-8.51) and 35.83 (15.57-82.43), respectively.

Table 33: Pooled diagnostic odds ratio (OR) of IGRAs for diagnosis of active TB in extrasanguinous fluids[1]

	Pooled diagnostic OR	95% CI	Inconsistency I^2 (%)	Number of studies
QFT-GIT	3.84	1.73-8.51	0	4
T-SPOT.TB	35.83	15.57-82.43	30.8	7

When performed with extrasanguinous fluids, the sensitivity of T-SPOT.*TB* was significantly higher (88%) compared with QFT-GIT (48%). Also, the number of indeterminate results was lower. Based on these data, authors suggested that the T-SPOT.*TB* assay is currently the best available extrasanguinous-based immunological method for the diagnosis of active TB. The authors therefore suggested that the T-SPOT.*TB* performed with extrasanguinous fluids could in low-incidence settings represent an improvement for the rapid diagnosis of active TB when combined with the standard methods of diagnosis[1].

The authors also pointed out that data are limited and that the existing data indicate that IGRAs have limited accuracy in diagnosing active TB when used with extrasanguinous samples.

4.2 Large-scale population screening for LTBI

Considerations

- Large-scale screening encompasses mass screenings (large-scale screening of whole population groups) and selective screening (screening of selected high-risk groups in a population performed at a large scale)[70].
- In EU countries, the aim of large-scale screening usually is to improve active-TB case-finding and subsequently treat active TB in population groups that have a considerably higher prevalence than average.
- In large-scale screening, a population is commonly mixed in terms of age and BCG vaccination status, and may be composed of individuals originating from high- or low-TB incidence setting as well as groups for which the general immune status should be taken into account.
- IGRAs could have an advantage by providing results after only one visit to the healthcare facility; however, a second visit is required in case of a positive test result.
- Limited evidence is available on the predictive value of IGRAs in large-scale screenings in low-TB incidence settings, and studies on specific risk groups with higher statistical power are needed.

Expert opinion

The decision to conduct large-scale screenings should be based on evidence of the cost-effectiveness of screenings in populations with a high risk.

The decision to use IGRAs alone or in combination with TST in the diagnosis of LTBI is based on the evidence and opinions presented in the above sections referring to population groups or situations. The PPV of the test will vary widely according to the risk of LTBI in the tested population.

4.3 Future research needs

Prospective studies on diagnostic accuracy

- More and larger prospective studies assessing the positive and negative predictive values of IGRAs for the diagnosis of LTBI (and, if possible, TST). Further studies are also needed for the diagnosis of active TB in different settings (low-, intermediate- and high-TB incidence settings) and unselected populations.
- More studies assessing IGRA accuracy and predictive value (and possible limitations) in children (and particularly in children less than five years old) and other high-risk groups such as immunocompromised populations.
- Value of IGRAs in BCG-vaccinated individuals and/or exposed to NTM.
- More studies assessing the ability of IGRAs to discriminate recent from remote LTBI.
- Studies assessing the impact of re-infection with *M. tuberculosis* on immune reactivity, as defined by IFN production in IGRAs.
- More studies addressing the reproducibility of IGRAs and the phenomenon of conversion/reversion of IGRA-results over time (serial testing) as well as after treatment for active TB and LTBI.
- Effect of blood incubation delay on IGRA accuracy and extent of indeterminate results.
- More studies assessing the accuracy of IGRAs when used with extrasanguinous fluids in order to diagnose active TB.
- Studies determining the accuracy of IGRAs in the diagnosis of extrapulmonary TB.
- There is the need to provide harmonised guidelines for prospective studies so investigators have clear definitions, including clinical phenotypes for collating results in order to reach meaningful conclusions with adequate statistical power.

Biological/immunological issues

- More studies to identify the biological basis for discordant results between TST and IGRAs.
- Research to develop IGRAs that incorporate new *M. tuberculosis*-specific antigens and alternative cytokines that would enhance sensitivity, allowing LTBI to be distinguished from active TB.
- Studies assessing which cells contribute to IFN-γ production once *M. tuberculosis* infection has been cleared, e.g. by appropriate drug treatment.

Programmatic issues

- Studies to evaluate the feasibility and cost of IGRAs in the diagnosis of LTBI and active TB in different settings and for different purposes (e.g. contact screening, serial testing of healthcare workers).
- Studies to evaluate the resources needed for the implementation of IGRAs.

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