การวินิจฉัยการติดเชื้อวัณโรคแฝง: การประเมินบทบาทของ QuantiFERON-TB ในยุคของเทคโนโลยีใหม่

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บทคัดย่อ

วัณโรคมีสาเหตุจากเชื้อแบคทีเรีย *Mycobacterium tuberculosis* (*Mtb*) ยังคงเป็นปัญหาท้าทายด้านสุขภาพทั่วโลก วัณโรคมีความชุกสูงในภูมิภาคเอเชียตะวันออกเฉียงใต้ แอฟริกา และแปซิฟิกฝั่งตะวันตก การระบุผู้ติดเชื้อวัณโรคแฝงเป็นสิ่งสำคัญในการป้องกันการแพร่เชื้อ โดยเฉพาะในกลุ่มที่มีความเสี่ยงสูง เช่น ผู้ติดเชื้อเอชไอวี QuantiFERON-TB (QFT) ได้ถูกพัฒนาเป็นชุดทดสอบเลือดสำหรับวินิจฉัยวัณโรคแฝงที่มีข้อได้เปรียบอย่างมีนัยสำคัญ มีข้อดีกว่าการทดสอบผิวหนังแบบดั้งเดิม บทความนี้มุ่งบรรยายความสำคัญของการทดสอบ QFT ในการวินิจฉัยวัณโรค โดยอาศัยข้อมูลที่ได้จากการสำรวจความก้าวหน้าล่าสุดและอภิปรายถึงโอกาสในอนาคตของการทดสอบ QFT เนื้อหาในบทความได้ทบทวนวรรณกรรมโดยมุ่งเน้นที่ประโยชน์และความท้าทายของการทดสอบ QFT สำหรับการวินิจฉัยวัณโรค แสดงให้เห็นและเน้นย้ำถึงศักยภาพของแอนติเจนใหม่ การบูรณาการตัวบ่งชี้ทางชีวภาพและการประยุกต์ใช้อย่างกว้างขวางในสภาพแวดล้อมที่มีทรัพยากรจำกัดและประชากรมีความเสี่ยงสูง เมื่อเปรียบเทียบกันแล้ว ชุดการทดสอบ QFT เป็นเครื่องมือที่มีคุณค่าสำหรับการควบคุมและกำจัดวัณโรค การพัฒนาในอนาคตควรมุ่งเน้นไปที่การค้นหาแอนติเจนและตัวบ่งชี้ทางชีวภาพใหม่ การบูรณาการ QFT กับเครื่องมือวินิจฉัยอื่นและการเพิ่มประสิทธิภาพการใช้งานในสภาพแวดล้อมที่มีทรัพยากรจำกัดและกลุ่มเสี่ยง จะเห็นได้ว่าการทดสอบ QFT มีส่วนในการเปลี่ยนแปลงการวินิจฉัยวัณโรคแฝงได้อย่างมาก ซึ่งในท้ายที่สุดจะเป็นส่วนช่วยควบคุมและกำจัดการติดเชื้อวัณโรคในระยะยาว

คำสำคัญ: การทดสอบ QuantiFERON-TB การติดเชื้อวัณโรคแฝง อินเตอร์ฟีรอน-แกมมา เชื้อวัณโรค

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Latent Tuberculosis Infection Diagnosis: Re-evaluating the Role of QuantiFERON-TB in the Era of New Technologies

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) remains a global health challenge, with high prevalence in Southeast Asia, Africa and the Western Pacific. Identification of latent tuberculosis infection (LTBI) individually is essential for preventing disease progression, especially in high-risk groups, for instance, HIV-positive individuals. QuantiFERON-TB (QFT) has effectively emerged as blood test for LTBI diagnosis, offering significant advantages over the traditional tuberculin skin test (TST). This review highlights the strengths of QFT for TB diagnosis. The article explored recent advancements in the field and discussed the promising future of QFT. Comprehensive review, focusing on the benefits and challenges associated with QFT was thoroughly conducted. The review investigated the potential of novel antigens, biomarker integration and wider application in resource-limited settings and high-risk populations. Comparatively, QFT served as a valuable tool for TB control and elimination. Future research and development should involve discovering new antigens and biomarkers, integrating QFT with other diagnostic tools and adopting its use in resource-constrained settings and high-risk groups. QFT has revolutionized LTBI diagnosis. Recognizing its advancements can further enhance QFT role in TB management, ultimately infection control and elimination efforts.

Keywords: QuantiFERON-TB, Latent tuberculosis infection, Interferon-gamma, Mycobacterium tuberculosis

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Introduction

Million people globally have been affected by tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*), which remains a significant public health threat despite ongoing control efforts. The eradication program against TB faced persistent regional challenges. In 2022, estimated 10.6 million TB infected cases were reported, majority in Southeast Asia (46%), Africa (23%) and the Western Pacific (18%). Notably, 6.3% of these cases were HIV positive individuals, underlining the need for integrated programs to address co-infections.[1] One of the main challenges in TB control is the timely and accurate diagnosis of the infection. TB can affect various organs. The most common form is pulmonary TB, which affects the lungs and can be transmitted via respiratory droplets.[2]

Latent TB infection (LTBI), harbouring *Mtb* without active symptoms, affects a quarter of the global population with a 5-10% lifetime risk of progression.[3-5] QuantiFERON-TB (QFT), a blood test measuring the immune response to *Mtb* antigens, offers advantages like higher specificity than the traditional tuberculin skin test (TST). However, limitations include variability in test performance and the lack of a definitive link to TB progression.[6,7] Despite these challenges, QFT is a significant advancement in tackling LTBI, particularly in high-risk HIV patients in Thailand.

This review aims to provide an overview of immune response and immune effectors, the current status and future perspectives of QFT testing for the diagnosis of *Mtb* infection. This article discusses the principles, applications and challenges of QFT, as well as, the recent advances and innovations in the field. This article also highlights the gaps in knowledge and the areas of research that need further exploration to improve the utility and accuracy of the test.

Mechanism of T cell stimulation

Antigen presenting cells (APCs)

APCs, including macrophages and dendritic cells, are instrumental in initiating the immune response against *Mtb*. The APCs can take up *Mtb* antigens, process into small peptides, and present these fragments on the cells surface in conjunction with major histocompatibility complex (MHC) molecules.[16,17,34] This antigen presentation enables T cells to identify antigenic peptides as foreign substances, triggering a proper immune response.[16,17] Recent research has illuminated additional mechanisms through which APCs can activate *Mtb*-specific T cells. Macrophages can release exosomes, minuscule vesicles bound by a membrane that contain highly antigenic *Mtb* components. These exosomes can be internalised by T cells, stimulating both CD4+ and CD8+ T cell responses.[18,19]

Advancements in field of immunotherapy have been highlighted. The recent exemplification was the creation of artificial antigen presenting cell-mimicking scaffolds (APC-ms). These engineered scaffolds can be loaded with specific *Mtb* antigens and potentially adjusted to modulate the levels of T cell stimulation. This innovative technology offers the potential for personalised immunotherapies that can augment T cell ability to eliminate *Mtb* infection.[20,21]

MHC presentation

MHC molecules serve as a conduit between APCs and T cells, functioning as a platform for showcasing processed peptide fragments derived from *Mtb* antigens on the cell surface of APCs. The antigen presentation is pivotal for T cells to identify antigen fragments via T cell receptors (TCRs). Contrary to B cell receptors, which can bind directly to entire antigens, TCRs can only recognise specific peptide fragments associated with MHC molecules. This mechanism ensures that T cells can distinguish self and non-self antigens, thereby initiating specific immune response.[16,17]

Recent breakthroughs in MHC biology research have yielded invaluable insights into antiviral immune responses.[22,23] This knowledge is being harnessed to devise innovative cancer therapy targeting tumour neoantigens, unique protein fragments generated by tumour cells.[24-26]

Co-stimulatory signals

While the initial identification of the *Mtb* antigen by the TCRs is vital, it alone is insufficient for comprehensive T cell activation. Co-stimulatory signals, delivered via molecules such as CD28 on the T cell surface, are indispensable for full T cell activation. This dual-signal model underscores the necessity for both an antigen-specific signal (signal 1) and a co-stimulatory signal (signal 2) for the full activation of naive T cells. However, pre-activated T cells may only necessitate signal 1 for subsequent activation. This co-stimulatory interaction, particularly with CD28, is pivotal in promoting T cell proliferation and clonal expansion. Thereby enabling the immune system to effectively respond to the *Mtb* infection is proceeded.[27,28,35-37]

Recent investigations have delved into the role of co-stimulatory receptor signalling in CAR-T cell therapy, a variant of immunotherapy for cancer. These studies illustrate that the manipulation of co-stimulatory signals in CAR-T cells can markedly influence their overall functionality. Factors such as response kinetics, persistence, durability, and toxicity profiles can all be affected by co-stimulatory signalling, ultimately impacting the safety and anti-tumour efficacy of CAR T cells.[29,30]

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Differential T cell response and INF-gamma secretion

Pre-existing memory T cells (Tm cells) from prior *Mtb* exposure significantly influence the T cell response and INF-gamma secretion.[31] Compared to naive T cells encountering the *Mtb* antigen for the first time, Tm cells possess several advantages.[31] With a reduced activation threshold, Tm cells necessitate a less potent antigen signal for activation than naive T cells. This characteristic facilitates a quicker and more potent immune response upon *Mtb* re-exposure.[32]

Regarding enhanced migration and persistence, Tm cells demonstrate an increased ability to migrate to and persist within lymph nodes.[33] This strategic positioning enables them to swiftly encounter APCs presenting *Mtb* peptides, leading to a more effective immune response.[33] Moreover, Tm cells can endure for prolonged periods, offering enduring protection against *Mtb* re-infection.[31,38]

Overview of QuantiFERON-TB

QFT is a blood test that helps diagnose latent and active TB caused by *Mtb* complex organisms. The test measures the amount of interferon-gamma (IFN-gamma) that T cells release in response to particular Mtb antigens. Briefly, patient blood samples are collected and separated into four tubes: a nil control, a mitogen control, and two tubes containing different sets of *Mtb* antigens. The tubes are then incubated for 16 to 24 hours, and the amount of IFN-gamma in the plasma is measured by enzyme-linked immunosorbent assay (ELISA). The results are interpreted by comparing the IFN-gamma levels in the antigen tubes with the nil control and the mitogen control.[8,9]

Different modifications of QFT are available, each with a distinct *Mtb* antigen and various performance characteristics. The first generation QFT, called QuantiFERON-TB Gold (QFT-G), uses two peptides from two *Mtb* antigens: early secretory antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10. The second generation QFT, called QuantiFERON-TB Gold In-Tube (QFT-GIT), uses the same antigens as QFT-G but combines them into a single tube and adds a third peptide from another *Mtb* antigen called TB7.7. The third generation QFT, called QuantiFERON-TB Gold Plus (QFT-Plus), also uses the same antigens as QFT-GIT but divides them into two tubes. The first tube contains short peptides (TB1) designed to stimulate CD4+ T cells, while the other tube contains long peptides (TB2) designed to stimulate CD8+ T cells. The QFT-Plus test is expected to have higher sensitivity and specificity than the previous QFT tests, especially in immunocompromised populations.[7,8]

Antigens are substances that stimulate the immune system to produce antibodies or effector immune cells to eradicate infectious agents. Antigens used for QFT tests use synthetic peptides mimicing the proteins of *Mtb*, such as ESAT-6, CFP-10 and TB7.7.

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These antigens are more specific to *Mtb* than purified protein derivatives (PPD), which is derived from a mixture of many different mycobacteria and can cause false-positive results in people who have been vaccinated with BCG or exposed to NTM.[10]

Sensitivity is the ability of the test to correctly identify people who have LTBI or active TB infection. QFT has higher sensitivity than TST, ranging from 76.9% to 90.4%, depending on the type of test and the population studied.[11] TST has a sensitivity of about 70%, but it can vary depending on the dose and interpretation of the reaction.[9,12]

Specificity is the ability of the test to correctly identify people who do not have LTBI or active TB infection. QFT has higher specificity than TST, ranging from 98.1% to 99.0%, depending on the type of test and the population studied. TST has a specificity of about 59%, but it can be affected by BCG vaccination or NTM exposure.[9]

Reproducibility is the consistency of the test results when repeated under the same conditions. QFT has moderate to poor reproducibility, depending on the type of test and the population studied. T-SPOT.TB has the best reproducibility among QFT tests, followed by QFT-GIT, QFT-Gold, and QFT-Plus TST has poor reproducibility, as the reaction can vary depending on the technique, timing, and interpretation of the test.[7,13]

QFT has several advantages over TST, such as requiring only one visit, avoiding cross-reaction with BCG or NTM, and providing objective results. QFT-GIT has additional advantages over QFT-Gold, such as improved stability and standardisation of the blood samples. QFT-Plus has an additional advantage over QFT-GIT, such as enhanced detection of CD8+ T cells, which are important for controlling *Mtb* infection.[7,9]

The drawbacks of using the test for diagnosing LTBI or active TB infection are as follows: QFT tests have several disadvantages compared to TST, such as requiring blood draws, laboratory equipment, and trained personnel and having higher costs.[14,15] QFT-GIT has an additional disadvantage over QFT-Gold, such as higher cost. QFT-Plus has additional disadvantages over QFT-GIT, such as lower specificity and reproducibility. T-SPOT.TB has additional disadvantages over QFT-GIT, such as being more labour-intensive and variable.[9]

While TST remains the traditional method for latent TB detection, QFT offers several improvements. With more specificity, unlike TST, QFT is not swayed by past BCG vaccination or exposure to NTM, leading to more precise identification of those truly at risk for active TB infection. For boosted objectivity, TST relies on subjective interpretation of skin induration, introducing

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variability. QFT requires only one clinic visit for a blood draw, whereas TST often mandates two visits. This single-visit approach minimises patient inconvenience and reduces the risk of missed follow-up appointments, a common challenge with TST. For enhanced consistency, QFT exhibits reproducibility compared to TST, leading to more consistent diagnoses.[21,22] Additionally, QFT has a likelihood of a boosting effect, further improving result reliability. QFT drawbacks include costs due to specialised equipment and personnel, limited access in resource-constrained settings[23], stricter sample handling needs, and varying interpretation criteria across populations.[9,23]

Process of measuring interferon-gamma (IFN-gamma) production

The immune response against Mycobacteria involves a fascinating process where APCs enternalised these bacteria. These APCs then migrate to the lymph nodes, displayed antigenic peptides on their surface alongside MHC-II molecules. Upon recognising the MHC-II-antigen complex on the APC surface, the memory T cells become activated. Once activated, these memory T cells release a range of cytokines, including the potent IFN-gamma, marking the next phase of the immune response.

To measure IFN-gamma production, scientists have two main weapons in their arsenal. The *in vitro* blood test involves isolating peripheral blood mononuclear cells (PBMCs) from a patient's blood sample and exposing them to a specific Mycobacteria antigen. If memory T cells specific to the antigen are present, they will be activated and release IFN-gamma, which can be measured using an enzyme-linked immunosorbent assay (ELISA).

On the other hand, the TST is an *in vivo* method used to assess cell-mediated immunity (CMI) to Mycobacteria. In this test, a small amount of PPD, a mixture of proteins derived from Mycobacteria, is injected into the patient's skin. If the person has been infected with Mycobacteria in the past, memory T cells specific to the antigen will be present in the skin. Upon encountering PPD, these memory T cells become activated and release inflammatory mediators, including cytokines like IFN-gamma, causing a localised area of swelling and hardening at the injection site 48-72 hours later. The size of the induration is measured to assess the degree of CMI. Fig. 1 Overview of IFN-gamma measurement, which provides a visual depiction of this process.

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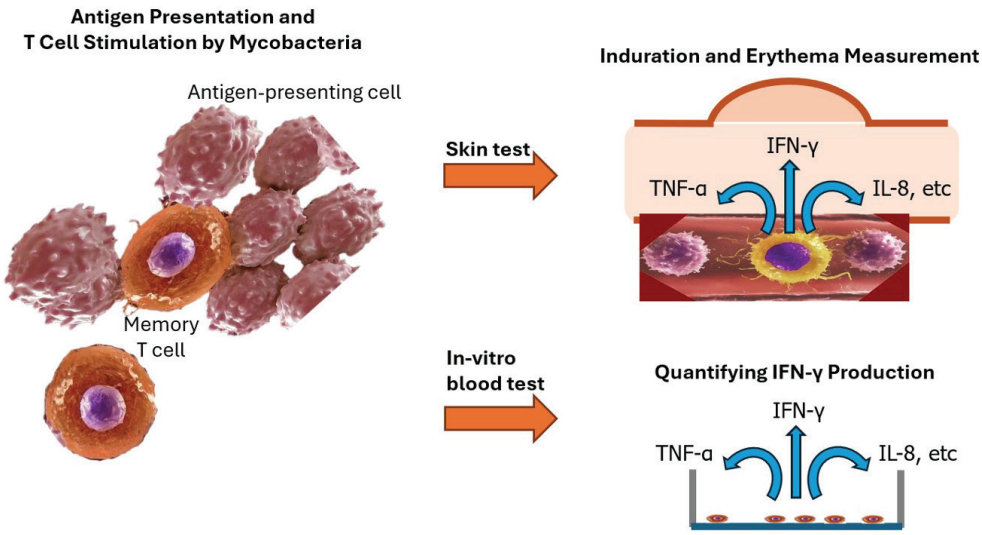

Fig. 1 Overview of IFN-gamma measurement

Each method has its strengths and weaknesses. Blood tests offer more specificity but are more complex and time-consuming. Skin tests are simpler but less specific and may not yield positive results in immunocompromised individuals.

Interpretation of test results

Negative result

A negative test outcome, marked by low INF-gamma secretion, could suggest no previous exposure to *Mtb* or a subdued immune response.[39] However, understanding a negative outcome does not conclusively exclude latent TB infection.[39] In certain scenarios, co-existing infections or immune-compromising conditions can lead to false-negative outcomes.[39]

Positive result

A positive test outcome, characterised by high INF-gamma secretion, indicates prior exposure to *Mtb.*[40] However, a positive outcome does not distinguish between latent TB infection and active TB disease.[40] Hence, additional tests, such as a chest X-ray or a sputum culture, are required to validate the diagnosis and ascertain the infection stage.[40]

Indeterminate result

An indeterminate outcome may arise if the test fails to provide valuable information about the probability of TB infection.[41] Indeterminate results could be attributed to various factors, such as an inadequate immune response or errors in the test procedure.[41] In these instances, the test may need to be

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repeated with a fresh blood sample, or alternative diagnostic methods may need to be explored depending on the specific circumstances.[41]

Timeline for QFT Gold+ (QFT-Plus) and other diagnostic tools

The timeline of QFT presents the evolution of different tests for latent TB infection, as shown in Table 1. The timeline table presents QFT, which is the first generation QFT in 2001, to the second generation QFT-G and QFT-GIT in 2005 and 2007, to the third generation QFT-Plus in 2017, which uses four antigens and measures both CD4+ and CD8+ T cell responses.

Table 1 Timeline table of QuantiFERON-TB Gold+ (QFT-Plus) and other diagnostic tools.

Year	Test	Event
1907	Tuberculin skin test (TST)[9]	TST was introduced by Charles Mantoux in 1907 as a method to detect TB infection by measuring the skin reaction to PPD injected intradermally.
2001	QuantiFERON-TB (QFT)[9]	QuantiFERON-TB (QFT)[9] was launched by Cellestis Limited (now QIAGEN). First, the interferon-gamma release assay (IGRA) was used to diagnose latent tuberculosis (LTBI). The kit measured interferon-gamma release in response to ESAT-6 antigen in whole blood.
2005	QuantiFERON-TB Gold (QFT-G)[9]	QuantiFERON-TB Gold (QFT-G) was launched by QIAGEN. This was the improved version of QFT using ESAT-6 and CFP-10 antigens for increased sensitivity and specificity.
2007	QuantiFERON-TB Gold In-Tube (QFT-GIT)[26]	QuantiFERON-TB Gold In-Tube (QFT-GIT) was launched by QIAGEN. This was the further improvement of QFT-G with a third antigen (TB7.7) and single blood collection tube for simpler procedure.
2008	T-SPOT.TB[27]	T-SPOT.TB was launched by Oxford Immunotec. The alternative IGRA for LTBI diagnosis by measuring interferon-gamma response from T cells stimulated by ESAT-6 and CFP-10 antigens in peripheral blood mononuclear cells.
2017	QuantiFERON-TB Gold Plus (QFT-Plus) [9]	QuantiFERON-TB Gold Plus (QFT-Plus) was launched by QIAGEN. The next generation QFT using four antigens (ESAT-6, CFP-10, and two Rv2654 peptides) to measure CD4+ and CD8+ T cell responses.

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Recent advances in QFT testing

The field of TB testing has made significant progress with the introduction of the QIAreach QuantiFERON-TB (QIAreach QFT) test.[42] This new test is an *in vitro* diagnostic test that uses a peptide cocktail to stimulate cells in heparinised whole blood.[42] The detection of interferon-gamma (IFN-gamma) by nanoparticle fluorescence is used to identify the *in vitro* responses to these peptide antigens, which are associated with *Mtb* infection.[42]

The QIAreach QFT test offers several potential benefits. It provides an objective readout with a minimum blood sample volume, which makes it a useful point-of-care screening test for *Mtb* infection in high-TB-burden, low-resource countries and for immunocompromised patients.[42] The test can deliver results in 3-20 minutes and can test up to 8 patients simultaneously on each eHub.[42] Moreover, it is a digital and connected system, which could facilitate contact tracing.[42]

The performance and accuracy of the QIAreach QFT test have been evaluated in several studies. A comparative performance evaluation of QIAreach QFT and the tuberculin skin test for the diagnosis of *Mtb*-infection in Vietnam showed that QIAreach QFT had a sensitivity of 98.5% and a specificity of 72.3%. The study also revealed that the QIAreach QFT test demonstrated a good performance in diagnosing *Mtb* infection and may offer technical and operational benefits over more complex IGRA ELISA-based assays.[42] The QIAreach QFT test represents a significant advancement in the field of TB testing, offering rapid, portable, and accurate testing that could be particularly beneficial in high-risk populations and resource-limited settings.

Comparison with other diagnostic tools

QFT is used to measure the immune response to the antigens of *Mtb*.[7] It is one of the methods used to diagnose latent or active TB infection, along with sputum smear microscopy, culture, nucleic acid amplification tests (NAATs), and chest radiography.[7] Each method has its advantages and disadvantages in terms of sensitivity, specificity, turnaround time, availability and cost.[7]

Compared to the tuberculin skin test, QFT has a higher specificity, particularly in people who have received the BCG vaccine.[43] The QuantiFERON-TB Gold In-Tube test (QFT-GIT) offers a more expedited turnaround time for results compared to traditional culture methods, eliminating the necessity for a secondary visit to interpret the outcomes. Nevertheless, the financial implications of QFT-GIT are considerably higher than those associated with the TST, potentially limiting its accessibility in resource-constrained environments. Furthermore, the sensitivity of QFT-GIT is inferior to Nucleic Acid Amplification Tests (NAATs) and culture methods, and it may yield indeterminate results in patients with compromised immune systems.[7]

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QFT is recommended for screening high-risk groups such as contacts of TB cases, healthcare workers, immigrants from high-prevalence countries, and people who are candidates for immunosuppressive therapy. It may also be useful for diagnosing *Mtb* infection in children and pregnant women who have limitations with other methods. However, QFT testing should not be used as the sole diagnostic tool for TB and should be interpreted in conjunction with clinical and radiological findings.[7]

Characteristics of QFT-Plus and other diagnostic tools for detecting *Mtb* are presented in Table 2. QFT-Plus, a diagnostic blood assay, quantifies interferon-gamma release from T cells upon *Mtb* antigenic stimulation, boasting a 94.1% sensitivity and 97.3% specificity. Advantageous for its single-visit requirement and objective outcomes without BCG vaccine cross-reactivity, they cannot differentiate active from latent TB.[7,9,30] The T-SPOT.TB assay is an alternative blood-based diagnostic tool that quantifies the population of T cells eliciting interferon-gamma production upon exposure to *Mtb* antigens. This assay demonstrates a sensitivity and specificity of 95.6% and 97.1%, respectively. However, it is noteworthy that the T-SPOT.TB assay incurs higher costs and demands more time for execution compared to the QuantiFERON-TB Gold Plus (QFT-Plus) test.[44-46]

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Table 2 Characteristics of QuantiFERON-TB Gold+ (QFT-Plus) and other diagnostics tools.

Method	Description	Sensitivity	Specificity	Advantages	Disadvantages
QuantiFERON-TB Gold+ (QFT-Plus)[7,9,44]	A blood test that measures the release of interferon-gamma from T cells stimulated by *Mtb* antigens	94.1%	97.3%	- Single visit required - Objective results - No cross-reactivity with BCG vaccine	- Unable to distinguish between active and latent TB - Requires a laboratory - Indeterminate results possible
T-SPOT.TB[44-46]	A blood test that measures the number of T cells that produce interferon-gamma when exposed to *Mtb* antigens	95.6%	97.1%	- Similar to QFT-Plus - Less affected by immunosuppression	- Similar to QFT-Plus - More expensive labour - Intensive than QFT-Plus
Tuberculin skin test (TST)[44,47,48]	A skin test that measures the delayed-type hypersensitivity response to intradermal injection of PPD	68.9%	59%	- Widely available - Low cost	- Two visits required - Subjective interpretation - Cross-reactivity with BCG vaccine and NTM - Boosting effect
Sputum smear microscopy[49-51]	A microscopic examination of sputum samples stained with Ziehl-Neelsen or auramine-rhodamine for acid-fast bacilli	50-80%	95-98%	- Rapid and simple - Low cost	- Low sensitivity, especially in HIV-positive patients - Cannot differentiate Mtb from other mycobacteria

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Table 2 Characteristics of QuantiFERON-TB Gold+ (QFT-Plus) and other diagnostics tools. (Cont.)

Method	Description	Sensitivity	Specificity	Advantages	Disadvantages
Culture[49,52,53]	A method of growing *Mtb* from clinical specimens, such as sputum, urine, or tissue, on solid or liquid media	80-85%	99%	- High sensitivity and specificity - Allows for drug susceptibility testing	- Slow (2-6 weeks) - Requires biosafety level 3 laboratory - Risk of contamination
Nucleic acid amplification tests (NAATs)[7,9]	A group of molecular tests that detect the genetic material of *Mtb* from clinical specimens	95%	98%	- Rapid (less than 2 hours) - High sensitivity and specificity - Can detect rifampin resistance	- Expensive - Requires sophisticated equipment and trained personnel - Prone to false-positive results due to contamination
Pleural fluid analysis[54]	A method of examining the fluid collected from the pleural space for biochemical, microbiological, and cytological characteristics	Variable	Variable	- Useful for differentiating transudate from exudate - Can detect acid-fast bacilli, mycobacterial DNA, or antigens	- Invasive procedure - Low sensitivity for acid-fast bacilli smear and culture - False-negative results possible

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TST is a skin test that measures the delayed-type hypersensitivity response to intradermal injection of PPD. It has a sensitivity of 68.9% and a specificity of 59%. It is widely available and inexpensive. However, TST may cross-reaction with the BCG vaccine and NTM.[44,47,48] Sputum smear microscopy is rapid, simple, and low cost but has a sensitivity of only 50-80% and a specificity of 95-98%. It cannot differentiate *Mtb* from other mycobacteria and has low sensitivity, especially in HIV-positive patients.[49-51] Culture is a method of growing *Mtb* from clinical specimens, such as sputum, urine, or tissue, on solid or liquid media. It has a sensitivity of 80-85% and a specificity of 99%. It provides high sensitivity and specificity.[49,52,53]

Nucleic acid amplification tests (NAATs) are molecular tests that detect the genetic material of *Mtb* from clinical specimens. NAAT offers a rapid diagnosis (less than 2 hours) of TB with high accuracy, 95% sensitivity, and 98% specificity.[7,9]

Pleural fluid analysis is a method of examining the fluid collected from the pleural space for biochemical, microbiological, and cytological characteristics. Its sensitivity and specificity are variable. However, it is an invasive procedure, has low sensitivity for acid-fast bacilli smear and culture, and false-negative results are possible.[54]

Challenges

QFT is commonly used for identifying latent TB infection. However, it has its own set of difficulties that affect its accuracy and usefulness. Some common issues include indeterminate results, which occur when the test fails to provide a valid response due to technical or biological factors.[14] Ongoing research refines QFT interpretation by establishing optimal cut-off values and accounting for co-morbidities and immunosuppression.[14]

The accuracy and reliability of QFT can be influenced by several factors that contribute to false-positive or false-negative results.[14] One of these factors is prior exposure to NTM, which are environmental bacteria that share some antigens with *Mtb* and can cross-reaction with the test. BCG vaccination is another factor as it induces an immune response to the test antigens, especially in young children and recent vaccines.

Future direction

Future direction and research priorities for QFT involve developing new TB-specific antigens and biomarkers that can improve the test's sensitivity and specificity, particularly in immunocompromised populations and children. Additionally, integrating QFT with other diagnostic tools such as molecular tests,

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chest radiography, and culture can enhance the accuracy and efficiency of TB diagnosis and management.[43]

QFT has the potential to impact the prevention and control of TB by identifying individuals with latent TB infection who are at high risk of developing active TB infection and who can benefit from preventive treatment. Moreover, QFT can reduce the transmission of TB by facilitating the detection and treatment of TB cases, especially those with drug-resistant TB.[43] Furthermore, QFT can improve the treatment outcomes of TB patients by monitoring their response to therapy and detecting treatment failure or relapse.[43]

The gaps and needs in the implementation and utilisation of QFT testing vary depending on the epidemiological and resource settings of different countries and regions.[9] Some of the common challenges include the high cost and complexity of the test, the limited availability and accessibility of the test kits and reagents, and the need for timely and reliable transportation and storage of blood samples.[9] To overcome these barriers, guideline updates and resource allocation support the adoption and scale-up of QFT in alignment with the WHO End TB Strategy and the Global Strategy for TB Research.[9]

Conclusion

TB is a significant global health concern arising from *Mtb* complex organisms. Early detection of *Mtb* infection in latent stages allows for preventative measures to be taken, preventing the development of active TB infection. QFT provides a novel diagnostic tool that can identify the immune response to specific antigens of *Mtb* in blood samples. Compared to traditional TST, QFT has several benefits, including requiring only one visit, providing an objective result, and being unaffected by prior BCG–vaccination. QFT is also useful for identifying high-risk individuals who may benefit from preventive treatment.

However, QFT faces some challenges, such as the inability to distinguish between latent and active TB infection, the requirement for laboratory facilities and trained personnel, and the occurrence of indeterminate results in some cases. Despite these challenges, QFT is a crucial tool for eliminating TB as it can help reduce the reservoir of LTBI and interrupt the transmission of *Mtb*.

In this review, the advancements and challenges in QFT cover its principles, methods, results and applications in various settings and populations. The new directions for future studies and improvement of QFT testing for optimal diagnosis and

management of *Mtb* infection are suggested. Our recommendations include enhancing the sensitivity and specificity of QFT, developing new biomarkers and assays for TB diagnosis and implementing QFT in resource-limited settings and high-risk groups.

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