QIAGEN

When accuracy matters

QuantiFERON®-TB Gold by QIAGEN

The most accurate test for TB infection

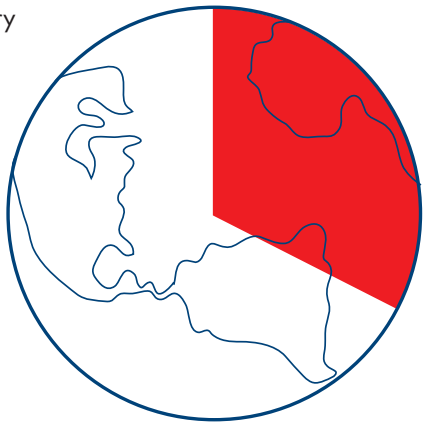
Sample to Insight

TB is one of the deadliest infectious diseases, but it can be prevented

Tuberculosis (TB) is a contagious infection responsible for more deaths annually than any other infectious disease, including HIV/AIDS. One third of the world's population is believed to be infected with *Mycobacterium tuberculosis*, the pathogen that causes TB (1). Individuals with latent TB infection (LTBI) show no symptoms. They may carry the infection for months or even years, and they are at risk for developing active and contagious TB disease.

The World Health Organization (WHO) acknowledges that to fight TB effectively, the accurate identification and treatment of LTBI as well as active TB disease are vital (1).

Unfortunately, outdated TB testing methods that are still commonly in use, such as the tuberculin skin test (TST), are not always sufficient to accurately identify true TB infection. This is especially true in high-risk populations such as the immunosuppressed, and in groups where the limitations of the TST are well-recognized, such as BCG-vaccinated individuals (2–3).



1/3 of the world is infected with latent TB

Together we can defeat TB

A test that accurately identifies TB infection is critical to reducing the global TB burden. Moreover, in times of increasing global migration, international borders are not sufficient to prevent the spread of TB. Prioritizing and testing those at greatest risk for infection is essential to preventing TB transmission (Table 1).

Table 1. The WHO recommends LTBI testing and treatment for populations at the highest risk (1)

Those most vulnerable to TB progression	Other prioritized at-risk populations
Contacts of pulmonary TB cases	Healthcare workers
People living with HIV	Prisoners
Patients initiating TNF-α treatment	Immigrants
Patients receiving dialysis	Individuals in congregate settings
Organ or hematologic transplantation patients	Illicit drug users

Newer TB blood tests, known as Interferon-Gamma Release Assays (IGRAs), address the limitations of the century-old tuberculin skin test (TST) and are finding widespread acceptance in the market. IGRAs detect TB infection by measuring the release of interferon-gamma (IFN-γ) from patient T cells after stimulation of a whole blood sample with highly specific TB antigens.

The US Centers for Disease Control (CDC) guidelines recommend the use of IGRAs in all situations in which the TST was historically used to detect TB infection. Moreover, IGRAs are the preferred test for persons who are BCG-vaccinated or are unlikely to return for TST reading (4).

Trust QuantiFERON-TB Gold

QuantiFERON-TB Gold (QFT®) is the most clinically tested and proven IGRA available (5). More than 30 million QFT tests have been sold across 130+ countries, including more than 7 million in 2015. Trust the only blood test for TB infection that offers:

- The most accurate and reproducible results for TB infection
- The confidence of more than 1200 clinical and scientific studies
- Convenient and objective ELISA technology
- Single visit testing

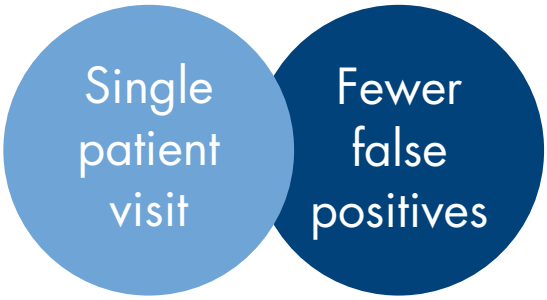
For detection of TB infection, accuracy matters

QFT is a simple, fresh blood test that produces more accurate results than the century-old skin test.

Unlike the TST, QFT is **not** affected by Bacille Calmette-Guérin (BCG) vaccination.

TST Challenges	QFT Solutions
Specificity as low as 59% in BCG-vaccinated patients (6)	>99% specific, nearly eliminating false positives and providing peace of mind for patients and physicians
Low sensitivity can cause missed true positives, putting contacts at risk (7)	Higher sensitivity than the TST, enabling truly infected patients to be identified and to receive appropriate antibiotic therapy
False positives from cross-reaction with the BCG vaccine and other environmental mycobacteria (2)	Unaffected by the BCG vaccine and most non-TB mycobacteria, reducing unnecessary antibiotic treatments

QFT is the simple, cost-effective solution for TB infection screening

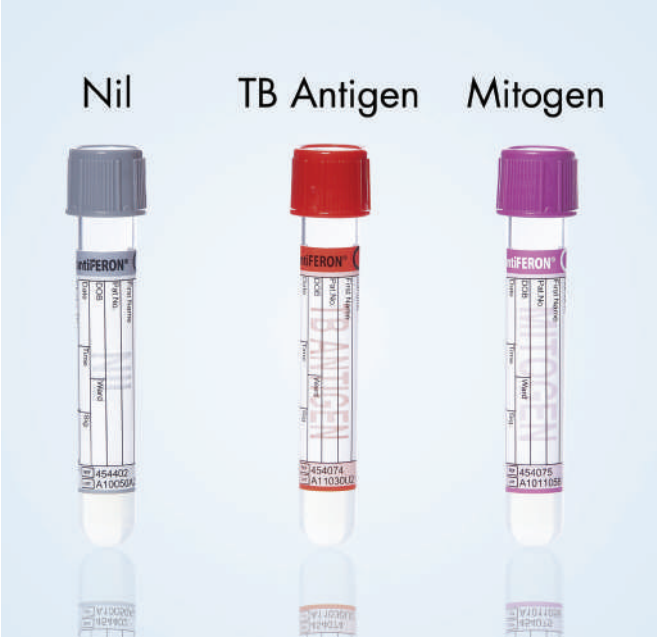


TST Challenges	QFT Solutions
High false positive rate causes unnecessary additional testing and costly treatment (6)	Low false positive rate reduces the cost and burden of unnecessary antibiotic treatment
High program costs from second visits, unnecessary x-rays and treatment (8–9)	Consistently shown to be more cost-effective in screening situations
Requires return visit to read the TST reaction	Results are sent directly to the physician, eliminating return visits for patients who test negative and encouraging follow-up for patients who test positive

You can lift the costly burden that inaccurate TB screening results place on your practice and on your patients. QFT produces fewer false positive results than the tuberculin skin test. QFT is also widely covered by Medicare, Medicaid and private insurance.

Modern and objective lab results using QFT technology

QFT is the industry-leading IGRA for TB detection. QFT uses unique blood collection tubes that enable immediate exposure of viable blood lymphocytes to highly specific TB antigens and test controls coated on the inner surface of the tubes. Antigen exposure produces a quantifiable immune response to aid in the diagnosis of TB infection.



The QFT advantage – three tubes, one clear result

Nil – negative control to adjust for background IFN-γ

TB Antigen – to detect the $CD4^+$ T cell responses to TB antigens

Mitogen – positive control to confirm baseline immune status

QFT is the fastest and easiest IGRA on the market:

- Requires only 3 ml of whole blood – 1 ml in each tube
- Optimized for speed and ease-of-use – no tedious lymphocyte isolation, subjective cell counting, diluting or culturing
- Results determined by objective ELISA analysis, rather than subjective counting of spots

Choose QFT for a flexible, convenient workflow

QFT employs whole blood collection to make T cell incubation simple and fast. After blood collection, the sample must be incubated, which can occur on-site or at the testing laboratory, providing your practice with complete flexibility and convenience.

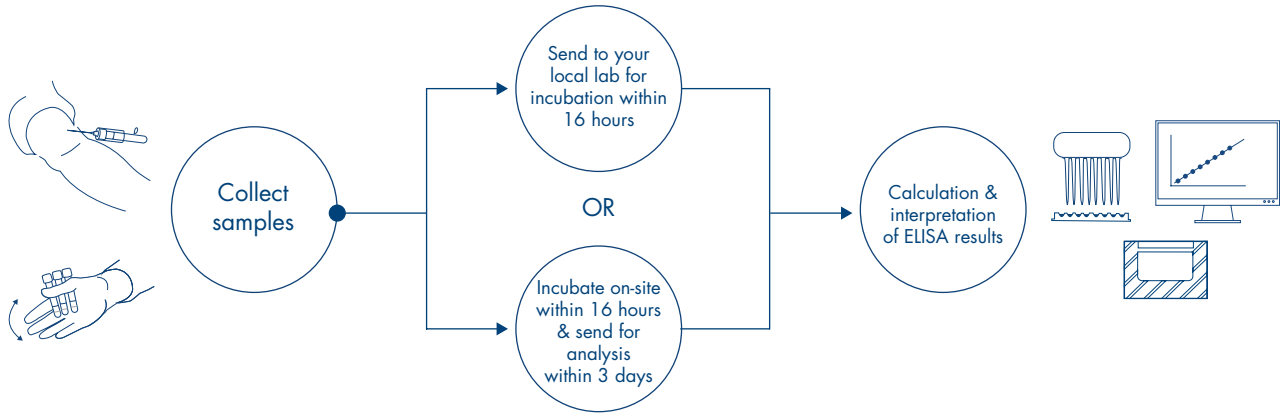


Figure 1. QFT provides a simple and rapid TB screening workflow.

The key to eradication is prevention – prevent TB in your community with QuantiFERON-TB Gold.

Improve your TB testing today –
Contact customer care at 1-800-426-8157 (USA) or visit **www.qiagen.com/shop**.

Not in the USA?
To find your local sales representative visit **www.qiagen.com/Goto/Customer-Care-Contact**.

References:

1. World Health Organization. (2015) Global tuberculosis report 2015. **www.who.int/tb/publications/global_report/en/**.
2. QuantiFERON-TB Gold (QFT) ELISA Package Insert. April 2015. 1075116 Rev. 02. Customers should consult current online version for their country at **www.QuantiFERON.com**.
3. Ponce de Leon, D., et al. (2008) Comparison of an interferon-gamma assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population. J. Rheumatol. **35**, 776.
4. Centers for Disease Control and Prevention. (2010) Updated guidelines for using interferon gamma release assays to detect Mycobacterium tuberculosis infection – United States, 2010. MMWR **59**(RR05), 1–25.
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8. Pareek, M. et al. (2013) Community-based evaluation of immigrant tuberculosis screening using interferon-γ release assays and tuberculin skin testing: observational, study and economic analysis. Thorax **68**, 230.
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QFT is approved by the US FDA. QFT is CE marked.

QFT is approved by FDA as an in vitro diagnostic aid for detection of *Mycobacterium tuberculosis* infection. It uses a peptide cocktail simulating ESAT-6, CFP-10, and TB7.7(p4) proteins to stimulate cells in heparinized whole blood.

Detection of IFN-γ by ELISA is used to identify in vitro responses to these peptide antigens that are associated with *M. tuberculosis* infection. QFT is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

The performance of the USA format of the QFT test has not been extensively evaluated with specimens from individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs, or those with other clinical conditions such as chronic renal failure or hematological disorders.

Up-to-date licensing information and product-specific disclaimers can be found at **www.QuantiFERON.com**.

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April 2019

QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA Package Insert

Σ 2 x 96 (622120)

Σ 20 x 96 (622822)

Version 1

IVD

For in vitro diagnostic use

The whole blood IFN-γ test measuring responses to ESAT-6 and CFP-10 peptide antigens

CE

REF 622120, 622822

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

R6 MAT 1083163

Contents

Intended Use

The QuantiFERON-TB Gold Plus (QFT-Plus) assay is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon-γ (IFN-γ) by enzyme-linked immunosorbent assay (ELISA) is used to identify in vitro responses to those peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

QFT-Plus is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.

Summary and Explanation of the Test

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* (MTB) complex organisms (*M. tuberculosis*, *M. bovis*, *M. africanum*), which typically spread to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease. A newly infected individual can become ill from tuberculosis within weeks to months, but most infected individuals remain well. Latent tuberculosis infection (LTBI), a noncommunicable asymptomatic condition, persists in some who might develop tuberculosis disease months or years later. The main purpose of diagnosing LTBI is to consider medical treatment for preventing tuberculosis disease. Until recently, the tuberculin skin test (TST) was the only available method for diagnosing LTBI. Cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection. However, some infected individuals, including those with a wide range of conditions hindering immune functions, but also others without these conditions, do not respond to tuberculin. Conversely, some individuals who are unlikely to have *M. tuberculosis* infection exhibit sensitivity to tuberculin and have positive TST results after vaccination with Bacille Calmette-Guérin (BCG) or infection with mycobacteria other than *M. tuberculosis* complex, or undetermined other factors.

LTBI must be distinguished from tuberculosis disease, a reportable condition which usually involves the lungs and lower respiratory tract but may also affect other organ systems. Tuberculosis disease is diagnosed from historical, physical, radiological, histological, and mycobacteriological findings.

QFT-Plus is a test for cell-mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most nontuberculous mycobacteria with the exception of *M. kansasii*, *M. szulgai*, and *M. marinum* (1). Individuals infected with MTB-complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.

The antigens used in QFT-Plus are a peptide cocktail simulating the proteins ESAT-6 and CFP-10. Numerous studies have demonstrated that these peptide antigens stimulate IFN-γ responses in T cells from individuals infected with *M. tuberculosis,* but generally not from uninfected or BCG-vaccinated persons without disease or risk for LTBI (1–32). However, medical treatments or conditions that impair immune functionality can potentially reduce IFN-γ responses. Patients with certain other mycobacterial infections might also be responsive to ESAT-6 and CFP-10, as the genes encoding these proteins are present in *M. kansasii*, *M. szulgai*, and *M. marinum* (1, 23). QFT-Plus is both a test for LTBI and a helpful aid for diagnosing *M. tuberculosis* complex infection in sick patients. A positive result supports the diagnosis of tuberculosis disease, but infections by other mycobacteria (e.g., *M. kansasii*) could also lead to positive results. Other medical and diagnostic evaluations are necessary to confirm or exclude tuberculosis disease.

QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB–complex–associated antigens, ESAT-6 and CFP-10. Whereas the TB1 tube contains peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from $CD4^+$ T-helper lymphocytes, the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from $CD8^+$ cytotoxic

T lymphocytes. In the natural history of MTB infection, $CD4^+$ T cells play a critical role in immunological control through their secretion of the cytokine IFN-γ. Evidence now supports a role for $CD8^+$ T cells participating in the host defense to MTB by producing IFN-γ and other soluble factors, which activate macrophages to suppress growth of MTB, kill infected cells, or directly lyse intracellular MTB (33–35). MTB-specific $CD8^+$ cells have been detected in subjects with LTBI and with active TB disease where IFN-γ producing $CD8^+$ cells may be frequently found (36–38). Moreover, ESAT-6 and CFP-10 specific $CD8^+$ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI, and may be associated with a recent MTB exposure (39–41). In addition, MTB-specific $CD8^+$ T cells producing IFN-γ have also been detected in active TB subjects with HIV co-infection (42, 43) and in young children with TB disease (44).

Principles of the assay

The QFT-Plus assay uses specialized blood collection tubes, which are used to collect whole blood. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens.

The QFT-Plus test is performed in two stages. First, whole blood is collected into each of the QFT-Plus Blood Collection Tubes, which include a Nil tube, TB1 tube, TB2 tube, and a Mitogen tube. Alternatively, blood may be collected in a single generic blood collection tube that contains lithium heparin or sodium heparin as the anticoagulant, and then transferred to QFT-Plus tubes.

The Mitogen tube is used with the QFT-Plus test as a positive control. This may be important where there is doubt as to the individual's immune status. The Mitogen tube also serves as a control for correct blood handling and incubation.

The QFT-Plus tubes are shaken to mix antigen with the blood and should be incubated at 37°C as soon as possible, and within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/ml) is

measured by ELISA. The QFT-Plus ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535). Results for the test sample are reported in International Units per ml (IU/ml) relative to the standard curve prepared by testing dilutions of the standard supplied with the kit.

Heterophile (e. g., human anti-mouse) antibodies in serum or plasma of certain individuals are known to cause interference with immunoassays. The effect of heterophile antibodies in the QFT-Plus ELISA is minimized by the addition of normal mouse serum to the Green Diluent and the use of F(ab')2 monoclonal antibody fragments as the IFN-γ capture antibody coated to the microplate.

A QFT-Plus assay is considered positive for an IFN-γ response to either TB antigen tube that is significantly above the Nil IFN-γ IU/ml value. The plasma sample from the Mitogen tube serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to improper specimen handling, incorrect filling/mixing of the Mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ. Elevated levels of IFN-γ in the Nil sample may occur with the presence of heterophile antibodies, or to intrinsic IFN-γ secretion. The Nil tube adjusts for background (e.g., elevated levels of circulating IFN-γ or presence of heterophile antibodies). The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB antigen tubes and Mitogen tube.

Time required for performing assay

The time required to perform the QFT-Plus ELISA is estimated below; the time of testing multiple samples when batched is also indicated:

37°C incubation of blood tubes:	16 to 24 hours
ELISA:	Approx. 3 hours for one ELISA plate
	(22 individuals)
	<1 hour labor
	Add 10 to 15 minutes for each extra plate

Components and Storage

Blood Collection Tubes*		200 tubes	Single Patient Pack	Dispenser Pack	HA 200 tubes	HA Single Patient Pack	HA Dispenser Pack
Catalog no.		622526	622222	622423	623526	623222	623423
Number of tests/pack		50	10	25	50	10	25
QuantiFERON Nil Tube (gray cap, white ring)	Nil	50 tubes	10 tubes	25 tubes			
QuantiFERON TB1 Tube (green cap, white ring)	TB1	50 tubes	10 tubes	25 tubes			
QuantiFERON TB2 Tube (yellow cap, white ring)	TB2	50 tubes	10 tubes	25 tubes			
QuantiFERON Mitogen Tube (purple cap, white ring)	Mitogen	50 tubes	10 tubes	25 tubes			
QuantiFERON Nil HA Tube (gray cap, yellow ring)	Nil HA				50 tubes	10 tubes	25 tubes
QuantiFERON TB1 HA Tube (green cap, yellow ring)	TB1 HA				50 tubes	10 tubes	25 tubes
QuantiFERON TB2 HA Tube (yellow cap, yellow ring)	TB2 HA				50 tubes	10 tubes	25 tubes
QuantiFERON Mitogen HA Tube (purple cap, yellow ring)	Mitogen HA				50 tubes	10 tubes	25 tubes
QFT-Plus Blood Collection Tubes Package Insert		1	1	1	1	1	1

* Not all product configurations are available in every country. Please refer to QIAGEN Customer Care (details on www.qiagen.com) for more information on what configurations are available for ordering.

ELISA components†	2 Plate Kit ELISA	Reference Lab Pack
Catalog no.	622120	622822
Microplate Strips (12 x 8 wells) coated with murine anti human IFN-γ monoclonal antibody	2 x 96-well Microplate Strips	20 x 96-well Microplate Strips
IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal)	1 x vial (8 IU/ml when reconstituted)	10 x vial (8 IU/ml when reconstituted
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml	10 x 30 ml
Conjugate 100x Concentrate, lyophilized (murine anti human IFN-γ HRP, contains 0.01% w/v Thimerosal)	1 x 0.3 ml (when reconstituted)	10 x 0.3 ml (when reconstituted)
Wash Buffer 20x Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml	10 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3′, 5,5′ Tetramethylbenzidine)	1 x 30 ml	10 x 30 ml
Enzyme Stopping Solution (contains 0.5M H_2SO_4)	1 x 15 ml	10 x 15 ml
QFT-Plus ELISA Package Insert	1	1

† See page 15 for precautions and hazard statements.

Materials Required but Not Provided

- 37°C ± 1°C incubator*. CO_2 not required
- Calibrated variable volume pipets* for delivery of 10 µl to 1000 µl with disposable tips
- Calibrated multichannel pipet* capable of delivering 50 µl and 100 µl with disposable tips
- Plate lid
- Microplate shaker*
- Deionized or distilled water, 2 liters
- Microplate washer (automated washer recommended)
- Microplate reader* fitted with 450 nm filter and 620 nm to 650 nm reference filter

* Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Specimen Storage and Handling

Blood collection tubes

- Store blood collection tubes at 4°C to 25°C.

Kit reagents

- Store kit reagents at 2°C to 8°C.
- Always protect Enzyme Substrate Solution from direct sunlight.

Reconstituted and unused reagents

For instructions on how to reconstitute reagents, please see page 26.

- The reconstituted kit standard may be kept for up to 3 months if stored at 2°C to 8°C.

  Note the date on which the kit standard was reconstituted.
- Once reconstituted, unused Conjugate 100x Concentrate must be returned to storage at 2°C to 8°C and must be used within 3 months.

  Note the date on which the conjugate was reconstituted.
- Working strength conjugate must be used within 6 hours of preparation.
- Working strength wash buffer may be stored at room temperature for up to 2 weeks.

Warnings and Precautions

For in vitro diagnostic use only.

Warnings

- A negative QFT-Plus result does not preclude the possibility of *M. tuberculosis* infection or tuberculosis disease: false-negative results can be due to stage of infection (e.g., specimen obtained prior to the development of cellular immune response), co-morbid conditions that affect immune functions, incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay, or other immunological variables.
- A positive QFT-Plus result should not be the sole or definitive basis for determining infection with *M. tuberculosis*. Incorrect performance of the assay may cause false-positive responses.
- A positive QFT-Plus result should be followed by further medical evaluation and diagnostic evaluation for active tuberculosis disease (e.g., AFB smear and culture, chest X-ray).
- While ESAT-6 and CFP-10 are absent from all BCG strains and from most known nontuberculous mycobacteria, it is possible that a positive QFT-Plus result may be due to infection by *M. kansasii*, *M. szulgai*, or *M. marinum*. If such infections are suspected, alternative tests should be performed.

Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: Handle human blood and plasma as if potentially infectious. Observe relevant blood and blood product handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

The following hazards and precautionary statements apply to components of the QuantiFERON-TB Gold Plus ELISA.

Hazard Statements

QuantiFERON Enzyme Stopping Solution

Contains: sulfuric acid. Warning! May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Enzyme Substrate Solution

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Green Diluent

Contains: trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate. Contains: tartrazine. Warning! May cause an allergic skin reaction. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Wash Buffer 20x Concentrate

Contains: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H -isothiazol-3-one (3:1). Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Precautionary Statements

Obtain special instructions before use. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If exposed or concerned: Get medical advice/attention. Immediately call a POISON CENTER or doctor/physician. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Store locked up. Dispose of contents/container to an approved waste disposal plant.

Further information

Safety Data Sheets: www.qiagen.com/safety

- Deviations from the *QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert* may yield erroneous results. Please read the instructions carefully before use.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.

- Important: Inspect vials prior to use. Do not use Conjugate or IFN-γ Standard vials that show signs of damage or if the rubber seal has been compromised. Do not handle broken vials. Take the appropriate safety precautions to dispose of vials safely. Recommendation: Use a vial de-crimper to open the Conjugate or IFN-γ Standard vials to minimize risk of injury from the metal crimp cap.
- Do not mix or use the Microplate Strips, IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QFT-Plus kit batches. Other reagents (Wash Buffer 20x Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be interchanged between kits providing the reagents are within their expiration periods and lot details recorded.
- Discard unused reagents and biological samples in accordance with Local, State, and Federal regulations.
- Do not use the QFT-Plus Blood Collection Tubes or ELISA kit after the expiration date.
- Correct laboratory procedures should be adhered to at all times.
- Make sure that laboratory equipment has been calibrated/validated for use.

Specimen Collection and Handling

QFT Plus uses the following collection tubes:

1. Quantiferon Nil Tubes (gray cap with white ring)
2. QuantiFERON TB1 Tubes (green cap with white ring)
3. QuantiFERON TB2 Tubes (yellow cap with white ring)
4. QuantiFERON Mitogen Tubes (purple cap with white ring)
5. QuantiFERON HA Nil Tubes (gray cap with yellow ring)
6. QuantiFERON HA TB 1 Tubes (green cap with yellow ring)
7. QuantiFERON HA TB2 Tubes (yellow cap with yellow ring)
8. QuantiFERON HA Mitogen Tubes (purple cap with yellow ring)

Antigens have been dried onto the inner wall of the blood collection tubes so it is essential that the contents of the tubes be thoroughly mixed with blood. For blood directly drawn into the QFT-Plus tubes, the QFT-Plus tubes must be maintained and transported at room temperature (22°C ± 5°C) and be transferred to a 37°C incubator as soon as possible and within 16 hours of collection. Alternatively, blood may be collected into a single lithium heparin or sodium heparin tube for storage prior to transfer to QFT-Plus and incubation. Blood specimens collected in lithium heparin or sodium heparin can be stored up to 16 hours at room temperature (17–25°C) followed by transfer to QFT-Plus tubes directly after collection. Blood specimens in lithium heparin or sodium heparin tubes may also be stored at 2–8°C for up to 48 hours prior to transfer to the QFT-Plus tubes. Refer to section "Blood collection in a single lithium or sodium heparin tube and then transfer to QFT-Plus Blood Collection Tubes."

Direct draw into QFT-Plus Blood Collection Tubes

1. Label tubes appropriately.

   Make sure each tube (Nil, TB1, TB2, and Mitogen) is identifiable by its label or other means once the cap is removed.

   It is recommended to record the time and date of blood collection.

2. For each patient, collect 1 ml of blood by venipuncture directly into each of the QFT-Plus Blood Collection Tubes. This procedure should be performed by a trained phlebotomist.

   Important note: Tubes should be between 17°C to 25°C at the time of blood filling.

   Standard QFT-Plus Blood Collection Tubes can be used up to an altitude of 810 meters above sea level. High Altitude QFT-Plus Blood Collection Tubes can be used between 1020 meters above sea level to an altitude of 1875 meters above sea level.

   As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling. This will ensure that the correct volume is drawn.

   - The black mark on the side of the tubes indicates the validated range of 0.8 to 1.2 ml. If the level of blood in any tube is outside the range of the indicator mark, a new blood sample should be obtained. Under or over-filling of the tubes outside of the 0.8 to 1.2 ml range may lead to erroneous results.
   - If a "butterfly needle" is being used to collect blood, a "purge" tube should be used to ensure that the tubing is filled with blood prior to the QFT-Plus tubes being used.
   - If using QFT-Plus Blood Collection Tubes at an altitude higher than 810 meters, or if low blood draw volume occurs, users can collect blood with a syringe, and immediately transfer 1 ml to each of the 4 tubes. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus tubes and adding 1 ml of blood to each (to the center of the black mark on the side of the tube label). Replace the caps securely and mix as described below. Ensure each tube (Nil,

TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

3. Immediately after filling the tubes, shake them ten (10) times just firmly enough to make sure that the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.

   Important note: Tubes should be between 17°C–25°C at the time of shaking. Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

4. Following labeling, filling, and shaking, the tubes must be transferred to a 37°C ± 1°C incubator as soon as possible, and within 16 hours of collection. Prior to incubation, maintain and transport the tubes at room temperature (22°C ± 5°C). If QFT-Plus tubes are not incubated at 37°C directly after blood collection and shaking, invert the tubes to mix 10 times prior to incubation at 37°C.
5. Incubate the QFT-Plus tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO_2 or humidification.

Blood Collection into a single lithium or sodium heparin tube and then transfer to QFT-Plus Blood Collection Tubes

1. Blood may be collected in a single blood collection tube containing lithium or sodium heparin as the anticoagulant and then transferred to QFT-Plus Blood Collection Tubes. Only use lithium or sodium heparin as a blood anticoagulant because other anticoagulants interfere with the assay. Label tubes appropriately.

   It is recommended to label the tube with the time and date of the blood collection.

   Important: Blood collection tubes should be at room temperature (17–25°C) at the time of blood collection.

2. Fill a lithium or sodium heparin blood collection tube (minimum volume 5 ml) and gently mix by inverting the tube several times to dissolve the heparin. This procedure should be performed by a trained phlebotomist.

3. Hold time and temperature options for lithium or sodium heparin tubes prior to transfer and incubation in QFT-Plus Blood Collection Tubes (See Figures 1-3 Blood Collection Options).

   Option 1 – Lithium or Sodium Heparin Tube Room Temperature Storage and Handling

   Blood collected in lithium or sodium heparin tube must be maintained at room temperature (22°C ± 5°C) for no more than 16 hours from the time of collection prior to transfer to QFT Plus Blood Collection Tubes and subsequent incubation.

   Option 2 – Lithium or Sodium Heparin Tube Refrigerated Storage and Handling

   Important: Procedural steps a–d must be followed in sequence.

   a. Blood drawn into lithium or sodium heparin tube may be held at room temperature (17–25°C) up to 3 hours after blood collection.
   b. Blood drawn into lithium or sodium heparin tube may be refrigerated (2–8°C) for up to 48 hours.
   c. After refrigeration, lithium or sodium heparin tube must equilibrate to room temperature (17–25°C) prior to transfer to QFT-Plus Blood Collection Tubes.
   d. Aliquoted QFT-Plus Blood Collection Tubes should be placed in the 37°C incubator within 2 hours of blood transfer.

   If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after transfer to QFT-Plus Blood Collection Tubes and shaking, invert the tubes to mix 10 times prior to incubation at 37°C. Total time from blood draw to incubation in QFT-Plus Blood Collection Tubes should not exceed 53 hours.

4. Transfer of blood specimen from a lithium or sodium heparin tube to QFT-Plus Blood Collection Tubes:

   a. Label each QFT-Plus Blood Collection Tube appropriately.

      Ensure each tube (Nil, TB1, TB2, and Mitogen) is identifiable by its label or other means once the cap is removed. It is recommended to transfer the recorded time and date of blood collection from the lithium or sodium heparin tubes to the QFT-Plus Blood Collection Tubes.

b. Samples must be evenly mixed by gentle inversion before dispensing into QFT Plus Blood Collection Tubes.

c. Dispensing should be performed aseptically, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus Blood Collection Tubes and adding 1 ml of blood to each tube. Replace the tube caps securely and mix as described below. Ensure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

5. Mix tubes. Immediately after filling the QFT-Plus Blood Collection Tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.

   Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

6. Following labeling, filling and shaking, the tubes must be transferred to a 37°C ± 1°C incubator within 2 hours. If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after blood collection and shaking, invert the tubes to mix 10 times (10x) prior to incubation at 37°C. (See Figures 1–3, next page, for blood collection options).

7. Incubate the QFT-Plus Blood Collection Tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO_2 or humidification.

Draw into QFT Plus Blood Collection Tubes and hold at room temperature.

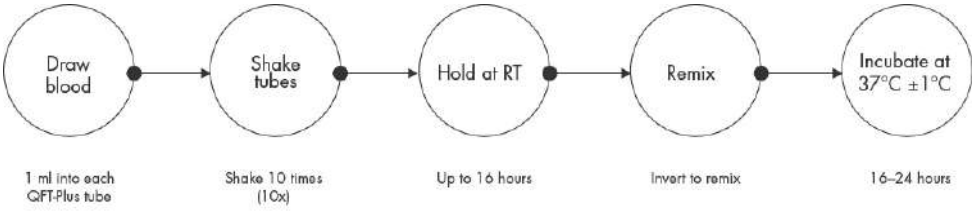


Figure 1. Blood collection option: Directly draw into QFT-Plus Blood Collection Tubes and hold at room temperature. The total time from blood draw in QFT-Plus Blood Collection Tubes to 37°C incubation must not exceed 16 hours.

Draw into lithium or sodium heparin tube and hold at room temperature.

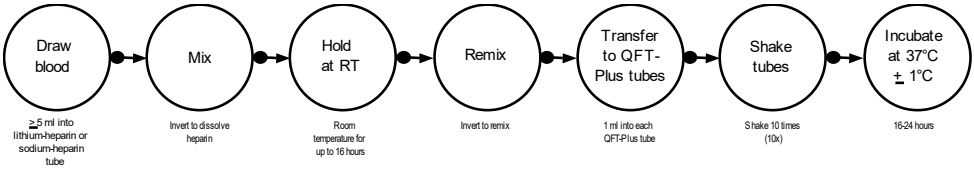


Figure 2. Blood collection option: Draw into lithium or sodium heparin tube and hold at room temperature.
The total time from blood draw in lithium or sodium heparin tube to 37°C incubation must not exceed 16 hours.

Draw into lithium or sodium heparin tubes and hold at 2–8°C.

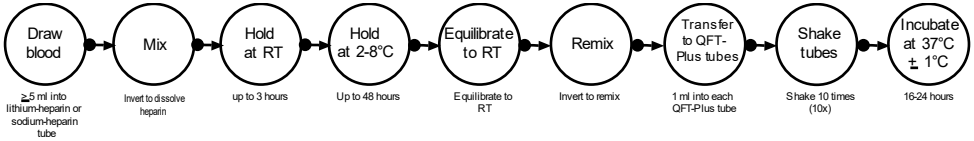


Figure 3. Blood collection option: Draw into lithium or sodium heparin tube and hold at 2–8°C.
The total time from blood draw in lithium or sodium heparin tube to 37°C incubation must not exceed 53 hours.

Directions for Use

Stage 1 – Incubation of blood and harvesting of plasma

Materials provided

- QFT-Plus Blood Collection Tubes (Refer to Section 3)

Materials required (but not provided)

- Refer to Section 3

Procedure

1. If the blood is not incubated immediately after collection, re-mixing of the tubes by inverting 10 times must be performed immediately prior to incubation.
2. Incubate the tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO_2 or humidification.
3. After incubation at 37°C, blood collection tubes may be held between 4°C and 27°C for up to 3 days prior to centrifugation.
4. After incubation of the tubes at 37°C, harvesting of plasma is facilitated by centrifuging the tubes for 15 minutes at 2000 to 3000 x RCF (*g*). The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged.

   Is it possible to harvest the plasma without centrifugation, but additional care is required to remove the plasma without disturbing the cells.
5. Plasma samples should only be harvested using a pipet.

   Important note: After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT-Plus ELISA plate, including when automated ELISA workstations are used.

Plasma samples can be stored for up to 28 days at 2°C to 8°C or, if harvested, below –20°C for extended periods.

For adequate test samples, harvest at least 150 µl of plasma.

Stage 2 – IFN–γ ELISA

Materials provided

- QFT-Plus ELISA kit (Refer to Section 3)

Materials required but not provided

- Refer to Section 3.

Procedure

1. All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C) before use. Allow at least 60 minutes for equilibration.
2. Remove strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until required.

   Allow at least 1 strip for the QFT-Plus standards and sufficient strips for the number of subjects being tested (refer to Figure 5). After use, retain frame for use with remaining strips.
3. Reconstitute the IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the vial. Mix gently to minimize frothing and ensure complete solubilization. Reconstitution of the standard to the stated volume will produce a solution with a concentration of 8.0 IU/ml.

   Important note: The reconstitution volume of the kit standard will differ between batches.

Use the reconstituted kit standard to produce a 1 in 2 dilution followed by a 1 in 4 dilution series of IFN-γ in Green Diluent (GD) (see Figure 4). S1 (Standard 1) contains 4.0 IU/ml, S2 (Standard 2) contains 1.0 IU/ml, S3 (Standard 3) contains 0.25 IU/ml, and S4 (Standard 4) contains 0 IU/ml (GD alone). The standards must be assayed at least in duplicate. Prepare fresh dilutions of the kit standard for each ELISA session.

Recommended procedure for duplicate standards
Label 4 tubes "S1", "S2", "S3", "S4."
Add 150 µl of GD to S1, S2, S3, S4.
Add 150 µl of the kit standard to S1 and mix thoroughly.
Transfer 50 µl from S1 to S2 and mix thoroughly.
Transfer 50 µl from S2 to S3 and mix thoroughly.
GD alone serves as the zero standard (S4).

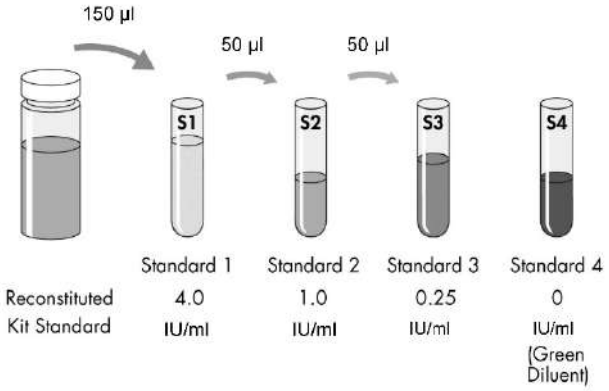


Figure 4. Preparation of standard curve.

4. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure complete solubilization of the conjugate.

   Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1. Conjugate Preparation). Return any unused Conjugate 100x Concentrate to 2°C to 8°C immediately after use. Use only Green Diluent.

Table 1. Conjugate Preparation

Number of strips	Volume of Conjugate 100x Concentrate	Volume of Green Diluent
2	10 µl	1.0 ml
3	15 µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

5. For plasma samples harvested from blood collection tubes and subsequently stored (refrigerated or frozen), mix samples before addition to the ELISA well.

   Important note: If plasma samples are to be added directly from the centrifuged QFT-Plus tubes, any mixing of the plasma should be avoided. At all times, take care not to disturb material on the surface of the gel.

6. Add 50 µl of freshly prepared working strength conjugate to the required ELISA wells using a multichannel pipet.
7. Add 50 µl of test plasma samples to appropriate wells using a multichannel pipet (refer to recommended plate layout in Figure 5). Finally, add 50 µl each of standards 1 to 4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 N	3 N	5 N	7 N	9 N	S1	S1	13 N	15 N	17 N	19 N	21 N
B	1 TB1	3 TB1	5 TB1	7 TB1	9 TB1	S2	S2	13 TB1	15 TB1	17 TB1	19 TB1	21 TB1
C	1 TB2	3 TB2	5 TB2	7 TB2	9 TB2	S3	S3	13 TB2	15 TB2	17 TB2	19 TB2	21 TB2
D	1 M	3 M	5 M	7 M	9 M	S4	S4	13 M	15 M	17 M	19 M	21 M
E	2 N	4 N	6 N	8 N	10 N	11 N	12 N	14 N	16 N	18 N	20 N	22 N
F	2 TB2	4 TB1	6 TB1	8 TB1	10 TB1	11 TB1	12 TB1	14 TB1	16 TB1	18 TB1	20 TB1	22 TB1
G	2 TB2	4 TB2	6 TB2	8 TB2	10 TB2	11 TB2	12 TB2	14 TB2	16 TB2	18 TB2	20 TB2	22 TB2
H	2 M	4 M	6 M	8 M	10 M	11 M	12 M	14 M	16 M	18 M	20 M	22 M

Figure 5. Recommended sample layout (22 tests per plate)

S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4)

1 N (Sample 1. Nil plasma), 1 TB1 (Sample 1. TB1 plasma), 1 TB2 (Sample 1. TB2 plasma), 1 M (Sample 1. Mitogen plasma)

8. Cover each plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute. Avoid splashing.
9. Cover each plate and incubate at room temperature (22°C ± 5°C) for 120 ± 5 minutes.

   Plates should not be exposed to direct sunlight during incubation.
10. During the incubation, dilute one part Wash Buffer 20x Concentrate with 19 parts deionized or distilled water and mix thoroughly. Sufficient Wash Buffer 20x Concentrate has been provided to prepare 2 liters of working strength wash buffer.

    Wash wells with 400 µl of working strength wash buffer for at least 6 cycles. An automated plate washer is recommended.

Thorough washing is very important to the performance of the assay. Make sure each well is completely filled with wash buffer to the top of the well for each wash cycle. A soak period of at least 5 seconds between each cycle is recommended.

Standard laboratory disinfectant should be added to the effluent reservoir and established procedures should be followed for the decontamination of potentially infectious material.

11. Tap plates face down on absorbent, low-lint towel to remove residual wash buffer. Add 100 µl of Enzyme Substrate Solution to each well, cover each plate, and mix thoroughly using a microplate shaker.
12. Cover each plate and incubate at room temperature (22°C ± 5°C) for 30 minutes.

    Plates should not be exposed to direct sunlight during incubation.
13. Following the 30-minute incubation, add 50 µl of Enzyme Stopping Solution to each well and mix.

    Enzyme Stopping Solution should be added to wells in the same order and at approximately the same speed as the substrate in step 11.
14. Measure the Optical Density (OD) of each well within 5 minutes of stopping the reaction using a microplate reader fitted with a 450 nm filter and with a 620 nm to 650 nm reference filter. OD values are used to calculate results.

Calculations and Test Interpretation

QFT Plus Analysis Software may be used to analyze raw data and calculate results. It is available from www.QuantiFERON.com. Please make sure that the most current version of the QFT-Plus Analysis Software is used.

The software performs a quality control assessment of the assay, generates a standard curve, and provides a test result for each subject, as detailed in the Interpretation of Results section.

As an alternative to using the QFT-Plus Analysis Software, results can be determined according to the following method.

Generation of standard curve

(If QFT-Plus Analysis Software is not used)

Determine the mean OD values of the kit standard replicates on each plate.

Construct a $\log_{(e)}$-$\log_{(e)}$ standard curve by plotting the $\log_{(e)}$ of the mean OD (y axis) against the $\log_{(e)}$ of the IFN-γ concentration of the standards in IU/ml (x axis), omitting the zero standard from these calculations. Calculate the line of best fit for the standard curve by regression analysis.

Use the standard curve to determine the IFN-γ concentration (IU/ml) for each of the test plasma samples, using the OD value of each sample.

These calculations can be performed using software packages available with microplate readers, and standard spreadsheet or statistical software (such as Microsoft® Excel®). It is recommended that these packages be used to calculate the regression analysis, the coefficient of variation (%CV) for the standards, and the correlation coefficient (*r*) of the standard curve.

Quality control of test

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:

- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate OD values must be ≤15%.
- Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
- The correlation coefficient (*r*) calculated from the mean absorbance values of the standards must be ≥0.98.

The QFT-Plus Analysis Software calculates and reports these quality control parameters.

If the above criteria are not met, the run is invalid and must be repeated.

The mean OD value for the Zero Standard (Green Diluent) should be ≤0.150. If the mean OD value is >0.150, the plate washing procedure should be investigated.

Interpretation of results

QFT-Plus results are interpreted using the following criteria (Table 2):

Important note: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QFT-Plus results.

Table 2. Interpretation of QFT-Plus results

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/Interpretation
≤8.0	≥0.35 and ≥ 25% of Nil value	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥ 25% of Nil value			
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	≥0.5	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	<0.5	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any			Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined

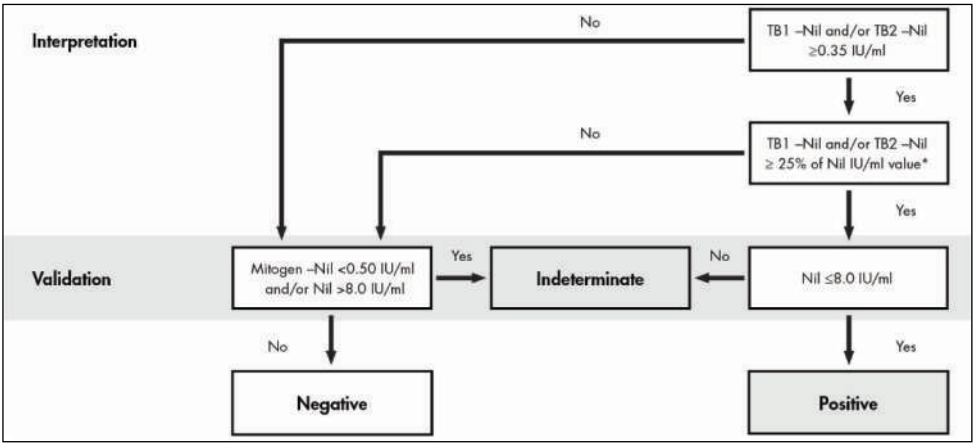
* Responses to the Mitogen positive control (and occasionally TB Antigens) can be outside the range of the microplate reader. This has no impact on test results. Values >10 ml are reported by the QFT-Plus software as >10 IU/ml.

† Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate in the QFT-Plus ELISA. If repeat testing of one or both replicates is positive, the individual should be considered test positive.

‡ Refer to the "Troubleshooting" section for possible causes.

§ In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

The magnitude of the measured IFN-γ level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. A positive TB response in persons who are negative to Mitogen is rare, but has been seen in patients with TB disease. This indicates the IFN-γ response to TB Antigen is greater than that to Mitogen, which is possible as the level of Mitogen does not maximally stimulate IFN-γ production by lymphocytes.



* For TB1 minus Nil or TB2 minus Nil to be valid, amount ≥25% of Nil IU/ml value must be from the same tube as the original ≥0.35 IU/ml result.

Figure 6. QFT-Plus interpretation flowchart

Limitations

Results from QFT-Plus testing must be used in conjunction with each individual's epidemiology, current medical status, and other diagnostic evaluations.

Individuals with Nil values greater than 8.0 IU/ml are classed as "Indeterminate" because a 25% higher response to the TB antigens may be outside the assay measurement range.

Unreliable or indeterminate results may occur due to:

- Deviations from the procedure described in this package insert
- Excessive levels of circulating IFN-γ or presence of heterophile antibodies
- Longer than 16 hours between drawing the blood specimen and incubation at 37°C. This is not applicable if using the lithium heparin or sodium heparin tube 2-8°C workflow.

Performance Characteristics

Clinical studies

As there is no definite standard test for LTBI, an estimate of sensitivity and specificity for QFT-Plus cannot be practically evaluated. Specificity of QFT-Plus was approximated by evaluating false-positive rates in the persons with low risk (no known risk factors) of tuberculosis infection. Sensitivity was approximated by evaluating groups of patients with culture-confirmed active TB disease.

Specificity

A study evaluating QFT-Plus specificity in 409 subjects was concluded. Demographic information and risk factors for TB exposure were determined using a standardized survey at the time of testing.

In a summary of findings from the 2 groups of patients with low risk (no known risk factors) for tuberculosis infection, the overall specificity of QFT-Plus was 97.6% (399/409) (Table 3 and Table 4).

Table 3. QFT-Plus specificity study results by study site

Study	Positive	Negative	Indeterminate	Specificity (95% CI)
Japan	4	203	0	98% (95–100%)
Australia	6	196	0	97% (94–99%)

Table 4. QFT-Plus specificity study results by TB antigen tube

Study	TB1	TB2	QFT-Plus
Positive	5	10	10
Negative	404	399	399
Indeterminate	0	0	0
Specificity (95% CI)	98.8% (97.2–99.6)	97.6% (95.6–98.8)	97.6% (95.6–98.8)

Sensitivity for active TB

While there is no definitive standard test for LTBI, a suitable surrogate is the microbiological culture of *M. tuberculosis* because patients with disease are by definition infected. TB suspects from 4 study sites in Australia and Japan who were subsequently confirmed to have *M. tuberculosis* infection by culture were tested to evaluate the sensitivity of QFT-Plus (Table 5 and Table 6). The patients had received less than 14 days of treatment prior to the collection of blood for QFT-Plus testing.

In a summary of findings from the 4 groups of *M. tuberculosis* culture–positive patients, the overall sensitivity of QFT-Plus for active TB disease was 95.3% (164/172). In the 4 groups, 159 patients were positive by both TB1 and TB2 tubes, 1 patient was positive by TB1 only, and 4 were positive by TB2 only. A total of 1.1% (2/174) of the results were indeterminate. The TB2 result correctly identified 1 culture–confirmed patient that would have been indeterminate (low Mitogen) by TB1 result alone (see Table 5 and Table 6).

Table 5. QFT-Plus sensitivity study results by study site

Study sites	Positive	Negative	Indeterminate	QFT-Plus sensitivity* (95% CI)
Japan site 1	36	7	0	84% (69–93)
Japan site 2	53	1	2	98% (90–100)
Japan site 3	54	0	0	100% (93–100)
Australia site	21	0	0	100% (84–100)

* Sensitivity is based on the total number of valid tests, excluding indeterminate results.

Table 6. QFT-Plus sensitivity study results by TB antigen tube

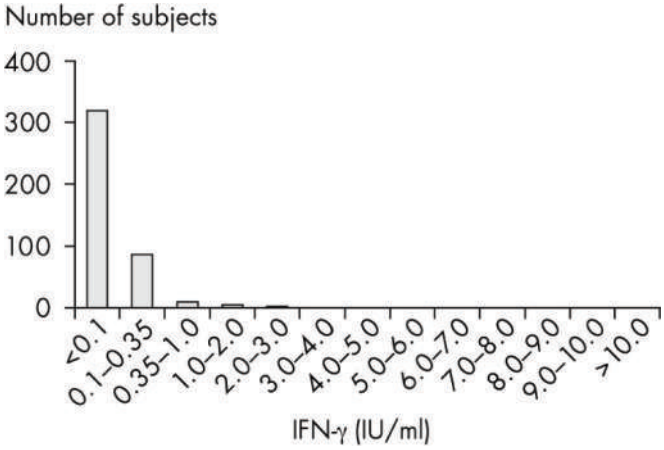
	TB1	TB2	QFT-Plus
Positive	160	163	164
Negative	11	9	8
Indeterminate	3	2	2
Sensitivity[†] (95% CI)	93.6% (88.8–96.7)	94.8% (90.3–97.6)	95.3% (90.9–97.9)

***** Sensitivity is based on the total number of valid tests, excluding indeterminate results.

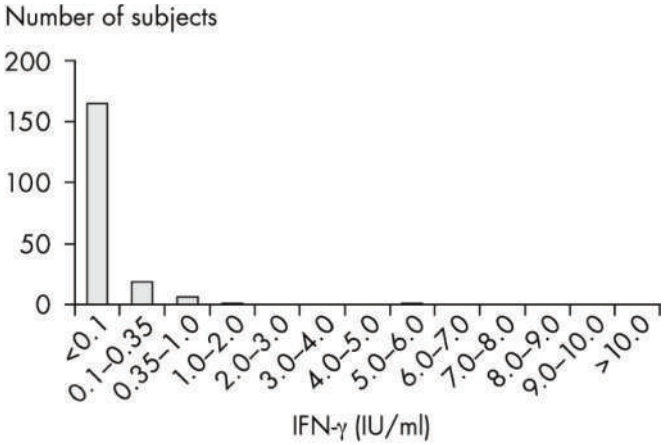
Observed response distributions – risk stratified

A range of IFN-γ responses to TB1, TB2, and control tubes were observed in clinical trials and stratified by risk of *M. tuberculosis* infection (Figures 7–9). The mixed risk group consists of subjects representative of a general testing population, including subjects with and without risk factors for TB exposure, and where active TB is unlikely (i.e., LTBI).

A



B



C

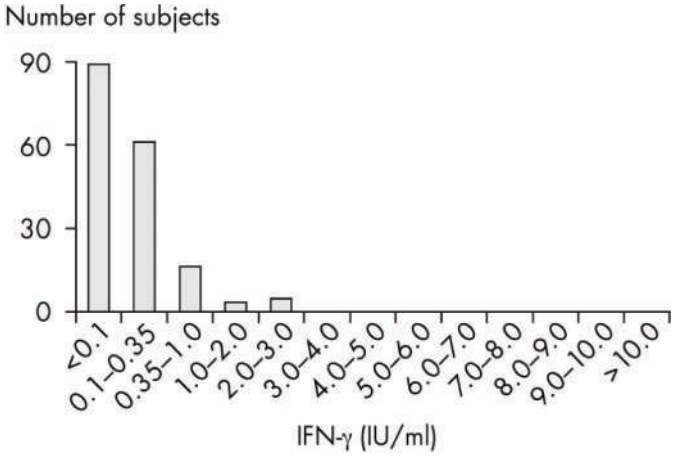


Figure 7. Distribution of Nil. A. Distribution of Nil values in a low-risk population (n=409). B. Distribution of Nil values in a mixed-risk population (n=194). C. Distribution of Nil values in a population with culture-confirmed *M. tuberculosis* infection (n=174).

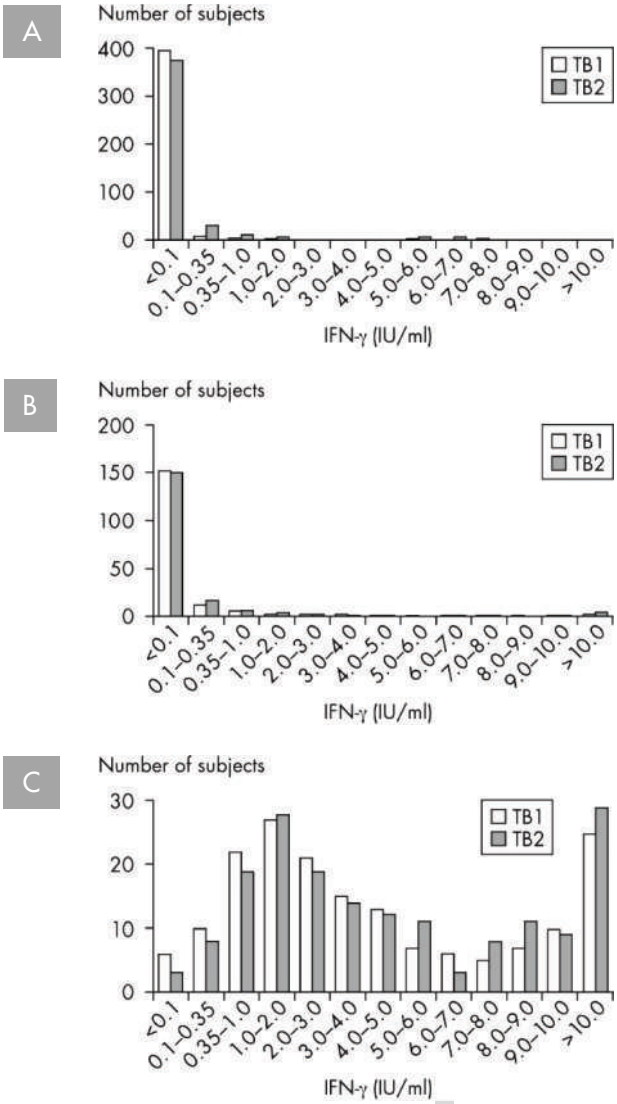


Figure 8. Distribution of TB1 and TB2 (nil subtracted). A. Distribution of TB1 and TB2 (nil subtracted) values in a low-risk population (n=409). B. Distribution of TB1 and TB2 (nil subtracted) values in a mixed-risk population (n=194). C. Distribution of TB1 and TB2 (nil subtracted) values in a population with culture-confirmed *M. tuberculosis* infection (n=174).

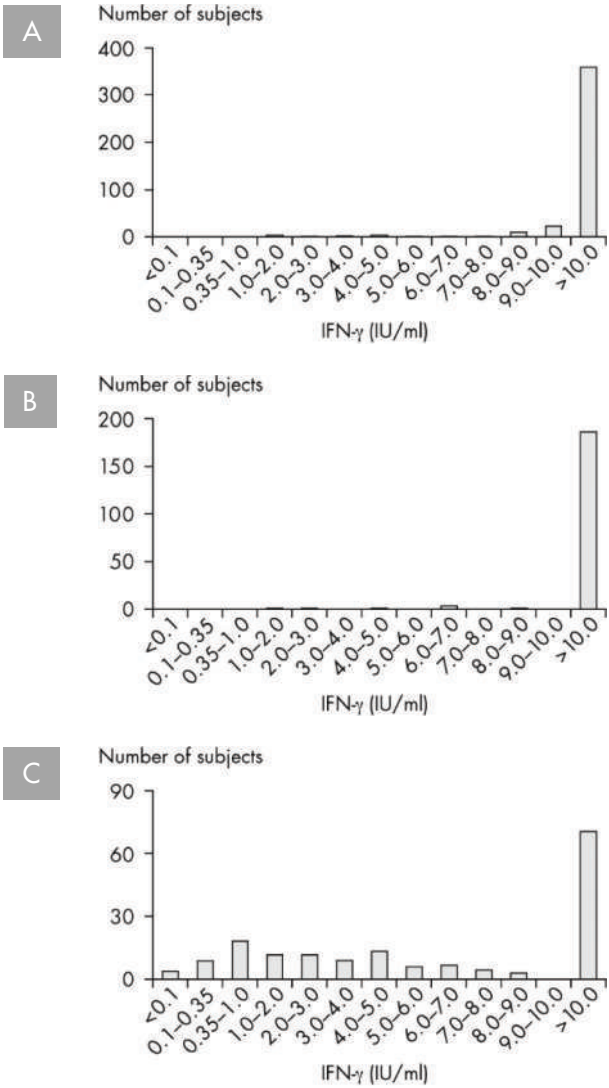


Figure 9. Distribution of Mitogen (nil subtracted). A. Distribution of Mitogen (nil subtracted) values in a low-risk population (n=409). B. Distribution of Mitogen (nil subtracted) values in a mixed-risk population (n=194). C. Distribution of Mitogen (nil subtracted) values in a population with culture-confirmed *M. tuberculosis* infection (n=169).

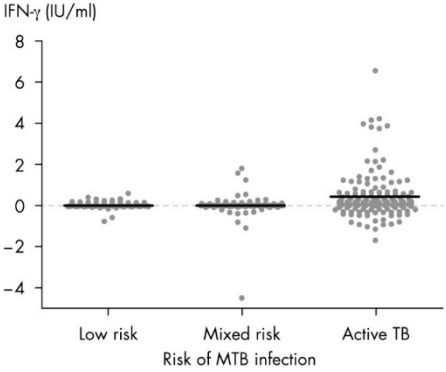


Figure 10. Observed difference between TB1 and TB2 values (nil subtracted), stratified by risk. Low-risk population (n=409), mixed risk population (n=189), and a population with culture confirmed *M. tuberculosis* infection (n=141). TB1 values were subtracted from TB2 values. Subjects with values for TB1 or TB2 of >10.0 IU/ml were excluded because they were outside the linear range of the assay.

Assay performance characteristics

The QFT-Plus ELISA has been demonstrated to be linear by placing 5 replicates of 11 plasma pools of known IFN-γ concentrations randomly on the ELISA plate. The linear regression line has a slope of 1.002 ± 0.011 and a correlation coefficient of 0.99 (Figure 11).

The limit of detection of the QFT-Plus ELISA is 0.065 IU/ml, and there is no evidence of a high-dose hook (prozone) effect with concentrations of IFN-γ up to 10,000 IU/ml.

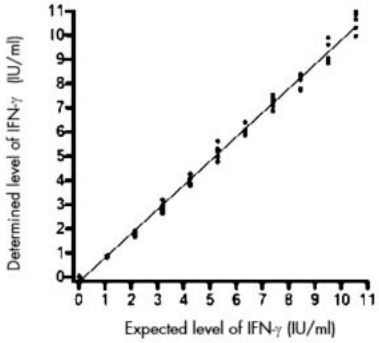


Figure 11. Linearity profile of QFT-Plus ELISA

Intra– and inter–assay imprecision (% CV) of the QFT-Plus ELISA was estimated by testing 20 plasma samples with varying IFN-γ concentrations in replicates of 3, in 3 different laboratories, on 3 nonconsecutive days, and by 3 different operators. Thus, each sample was tested 27 times in 9 independent assay runs. One sample was a nil control and had a calculated IFN-γ concentration of 0.08 IU/ml (95% CI: 0.07–0.09). Of the remaining 19 plasma samples, concentrations ranged from 0.33 (95% CI: 0.31–0.34) to 7.7 IU/ml (95% CI: 7.48–7.92).

Within run or intra-assay imprecision was estimated by averaging the %CVs for each test plasma containing IFN-γ from each plate run (n=9), and the imprecision ranged from 4.1 to 9.1%CV. The average within run covariance (±95% CI) was 6.6% ± 0.6%. The average of the zero IFN-γ plasma was 14.1% CV.

Total or inter-assay imprecision was determined by comparing the 27 calculated concentrations of IFN-γ for each test plasma. The inter-assay imprecision ranged from 6.6 to 12.3% CV. The overall average % CV (±95% CI) was 8.7% ± 0.7%. The zero IFN-γ plasma showed a 26.1% CV. This level of variation is to be expected because the calculated concentration of IFN-γ is low and variation around a low estimate of concentration will be larger than that for higher concentrations.

The reproducibility of the QFT-Plus test was determined using blood samples from 102 subjects with mixed risk factors for *M. tuberculosis* infection. Three different operators and laboratory conditions were assessed.

A total of 3 diagnostic determinations were made for each subject and 306 in total for all subjects. Overall, diagnostic reproducibility was 99% (95% CI: 97.2–99.7), where the diagnostic result was concordant for 303 of 306 determinations. The results of 3 subjects that were close to the cutoff accounted for all variation.

Diagnosis of LTBI

A number of studies have been published that demonstrate the performance of QFT, the precursor for QFT-Plus, in various populations at risk of infection with MTB. The principle findings of some selected studies are shown in Table 7.

Table 7. Selected published studies on QFT

Population/condition	Outcomes and findings	Total number of published studies
Pediatrics	Proven performance in children, including children less than 5 years of age (45–46) with higher accuracy than the ELISpot-based IGRA (8). Largest study to-date comparing QFT and TST in children from Vietnam, Philippines and Mexico supports the preferential use of QFT over TST for testing foreign-born children for LTBI (46). A limited contacts study shows better predictive value than TST in children (47) and 8-fold higher risk of progression to TB disease within two years among QFT converters compared to non-converters (48). QFT-negative/TST-positive discordance is high in BCG vaccinated children (46, 49), but there was no impact on Mitogen response in children under age 5 (49) and low indeterminate rates during routine screening of immigrant children (46).	152
Pregnancy	In a low-burden setting, QFT performs equally well in each trimester of pregnancy with comparable results to nonpregnant females, is much more specific, at least as sensitive, and may be a better predictor of disease progression than the TST (50). In a high-burden setting, QFT was more stable throughout pregnancy and more closely approximated the background LTBI prevalence compared to the TST, although the authors concluded that pregnancy affects both QFT and the TST (51).	6

Table continued on next page

Table 8. Selected published studies on QFT (continued)

Population/condition	Outcomes and findings	Total number of published studies
HIV/AIDS	Both IGRAs and TST are impacted by HIV infection, and the body of evidence suggests that caution should be taken when interpreting results in those with CD4+ counts <200 (52). QFT has been shown to be less affected than the ELISpot-based IGRA and TST (53–55). Single visit of IGRAs overcomes the TST issue of poor return rates in this population (53).	101
Immunosuppressive therapies	QFT is less impacted by immunosuppressive therapies than TST and correlates better with TB risk factors (23, 27). QFT has high sensitivity in patients with rheumatic disease (23; 56, 57) and higher specificity than TST, minimizing false positives and reducing unnecessary treatment that would occur with the TST (23, 57, 58).	112
Healthcare workers	Shown to be more specific with fewer false positives than the TST, and more cost-effective than the TST (59–62). Variability around the threshold is an expected finding in serial testing due to dichotomous cut-point and inherent variability of a biological test (63). Studies have shown higher conversion/reversion rates than TST in serial testing of low-risk healthcare workers (64, 65). The US CDC acknowledges that the lenient criterion to define IGRA conversion may produce more conversion than is observed with the more stringent quantitative criteria of the TST, and retesting strategies have been shown to be effective in managing the conversion/reversion phenomenon (65–68).	111
TB contacts	Higher PPV and NPV than the TST (47); convenience of single visit for those unlikely to return (63), better correlation to exposure (69), which is especially noted in BCG-vaccinated people and populations from BCG vaccinating countries (70, 71).	89
Transplantation	Has been shown to be at least as effective as TST, but less impacted by end-stage organ disease than the TST (22).	23

Table continued on next page

Table 9. Selected published studies on QFT (continued)

Population/condition	Outcomes and findings	Total number of published studies
Diabetes	Conflicting evidence from a small number of publications with limited numbers of subjects. A study from a low-burden area found that QFT sensitivity is not compromised by diabetes in TB patients (72). A study from Tanzania, a high-burden setting, suggesting a negative impact of diabetes on production of IFN-γ, failed to take into account confounders like HIV and helminth infections (73). In Vietnamese studies, 838 self-reported diabetics suspected of having TB due to abnormal CXRs or confirmed by culture to have active TB (n=128), QFT positivity was equal or greater than the TST cutpoints of 10 and 15 mm (74).	9
End-stage renal disease	QFT-positive results correlate with risk factors for TB better than TST and are less associated with BCG (75).	45
Migrants	Studies demonstrate QFT is unaffected by BCG and age unlike TST (74). QFT is shown to be the most cost-effective method (76). In low-burden settings, the majority of TB is coming from foreign born and from reactivation of latent TB after arrival (77). Largest study to-date comparing QFT and TST in immigrant children supports the preferential use of QFT over TST for testing foreign-born children for latent TB infection (46).	29

Technical Information

Indeterminate results

Indeterminate results are uncommon and may relate to the immune status of the individual being tested, but may also be related to a number of technical factors if the above instructions for use are not followed.

If technical issues are suspected with the reagent storage, blood collection, or handling of the blood samples, repeat the entire QFT-Plus test with a new blood specimen. Repeating the ELISA testing of stimulated plasmas can be performed if inadequate washing or other procedural deviation with the ELISA test is suspected. Indeterminate tests that result from low Mitogen or high Nil values would not be expected to change on repeat unless there was an error with the ELISA testing. Indeterminate results should be reported as such. Physicians may choose to redraw a specimen or perform other procedures as appropriate.

Clotted plasma samples

Should fibrin clots occur with long-term storage of plasma samples, centrifuge the samples to sediment clotted material and facilitate pipetting of plasma.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the technical information provided at www.QuantiFERON.com. For contact information, see back cover.

ELISA Troubleshooting

Nonspecific color development	
Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Cross-contamination of ELISA wells	Take care when pipetting and mixing sample to minimize risk.
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within three months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e) Mixing of plasma in QFT-Plus tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.
Low optical density readings for standards	
Possible cause	Solution
a) Standard dilution error	Ensure dilutions of the Kit Standard are prepared correctly as per this package insert.
b) Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c) Incubation temperature too low	Incubation of ELISA should be performed at room temperature (22°C ± 5°C).
d) Incubation time too short	Incubation of the plate with the conjugate, standards and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution is incubated on the plate for 30 minutes.

ELISA Troubleshooting

e) Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter between 620 and 650 nm.
f) Reagents are too cold	All reagents, with the exception of the Conjugate 100x Concentrate, must be brought to room temperature prior to commencing the assay. This takes approximately one hour.
g) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 months of the reconstitution date.

High background

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Incubation temperature too high	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.

Nonlinear standard curve and duplicate variability

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Standard dilution error	Ensure dilutions of the standard are prepared correctly as per this package insert.
c) Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
d) Inconsistent pipetting technique or interruption during assay set up	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

Product information and technical guides are available free of charge from QIAGEN, via your distributor, or by visiting www.QuantiFERON.com.

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Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
 2 × 96	Sufficient for 2 × 96 sample preparations
	Legal manufacturer
	CE-IVD marked symbol
IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalog number
GTIN	Global Trade Item Number
	Use by date
	Temperature limitation
	Consult instructions for use
	Do not reuse
	Keep away from sunlight
MAT	Material number
Rn	R is for revision of the Instructions for Use and n is the revision number

Contact Information

For technical assistance and more information, please call toll-free 00800-22-44-6000, see our Technical Support Center at www.qiagen.com/contact or contact one of the QIAGEN Technical Service Departments (see back cover or visit www.qiagen.com).

Abbreviated Test Procedure

Stage 1 – blood incubation

1. Collect patient blood into blood collection tubes and mix by shaking them ten (10) times just firmly enough to ensure that the entire inner surface of the tube has been coated with blood. This will dissolve antigens on tube walls.

2. Incubate tubes upright at 37°C ± 1°C for 16 to 24 hours.

3. Following incubation, centrifuge tubes for 15 minutes at 2000 to 3000 × *g* RCF (*g*) to separate the plasma and the red cells.

4. After centrifugation, avoid pipetting up and down or mixing the plasma by any means prior to harvesting. At all times, take care not to disturb the material on the surface of the gel.

Stage 2 – IFN–γ ELISA

1. Equilibrate ELISA components, with the exception of the Conjugate 100x Concentrate, to room temperature (22°C ± 5°C) for at least 60 minutes.

2. Reconstitute the kit standard to 8.0 IU/ml with distilled or deionized water. Prepare four (4) standard dilutions.

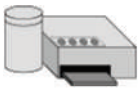
3. Reconstitute freeze-dried Conjugate 100× Concentrate with distilled or deionized water.

4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.

5. Add 50 µl of test plasma samples and 50 µl standards to appropriate wells. Mix using shaker.

6. Incubate for 120 ± 5 minutes at room temperature.

7. Wash wells at least 6 times with 400 µl/well of wash buffer.



8. Add 100 µl Enzyme Substrate Solution to wells. Mix using shaker.

9. Incubate for 30 minutes at room temperature.

10. Add 50 µl Enzyme Stopping Solution to all wells. Mix using shaker.

11. Read results at 450 nm with a 620 to 650 nm reference filter.

12. Analyze results.

Significant Changes

Section	Page	Change(s)
Various	Various	Added instructions related to the use of lithium heparin or sodium heparin tube
Various	Various	Added instructions related to 2–8°C blood collection workflow
Various	Various	Plate lid is now a material that is required but not provided

Handbook Revision History

Document	Changes
R6 04/2019	Lithium heparin/Sodium heparin changes New work instructions for 2–8°C blood collection workflow Plate lids removed from QF Plates

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Notes

Notes

August 2019

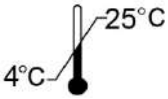
QuantiFERON®-TB Gold Plus (QFT®-Plus) Blood Collection Tubes Instructions for Use

Σ 50 (622526, 623526)

Σ 25 (622423, 623423)

Version 1

IVD



For in vitro diagnostic use

For use with QuantiFERON-TB Gold Plus ELISA or LIAISON® QuantiFERON-TB Gold Plus System

CE

REF 622526, 622423, 623526, 623423

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

R7 MAT 1085208

Contents

Blood Collection Tubes

Blood collection tubes		200 tubes	100 tubes
Catalog no.		622526	622423
QuantiFERON Nil Tube (gray cap, white ring)	Nil	50 tubes	25 tubes
QuantiFERON TB1 Tube (green cap, white ring)	TB1	50 tubes	25 tubes
QuantiFERON TB2 Tube (yellow cap, white ring)	TB2	50 tubes	25 tubes
QuantiFERON Mitogen Tube (purple cap, white ring)	Mitogen	50 tubes	25 tubes
QFT-Plus Blood Collection Tubes Package Insert		1	1

High Altitude (HA) Blood Collection Tubes (for use between 1020 and 1875 meters)		200 tubes	100 tubes
Catalog no.		623526	623423
QuantiFERON HA Nil Tube (gray cap, yellow ring)	Nil	50 tubes	25 tubes
QuantiFERON HA TB1 Tube (green cap, yellow ring)	TB1	50 tubes	25 tubes
QuantiFERON HA TB2 Tube (yellow cap, yellow ring)	TB2	50 tubes	25 tubes
QuantiFERON HA Mitogen Tube (purple cap, yellow ring)	Mitogen	50 tubes	25 tubes
QFT-Plus Blood Collection Tubes Package Insert		1	1

Important note: Altitude affects the blood collection volume of a tube. Use standard QFT-Plus Blood Collection Tubes between sea level and 810 m (2650 ft). Use High-Altitude (HA) tubes at altitudes between 1020 m (3350 ft) and 1875 m (6150 ft). If using QFT-Plus Blood Collection Tubes outside these altitude ranges, or if low blood-draw volume occurs, collect blood using alternate collection methods described below. The blood collection tubes supplied are for use only with the QFT-Plus ELISA or the LIAISON® QuantiFERON-TB Gold Plus System (REF: 311010), and the following instructions relate solely to the use of QFT-Plus Blood Collection Tubes.

Antigens have been dried onto the inner wall of the blood collection tubes, so it is essential to thoroughly mix the contents of the tubes with the blood. The tubes must be transferred to a

37°C incubator as soon as possible and within 16 hours of blood collection. Follow the procedures below for optimal results.

Precautions

For in vitro diagnostic use only.
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs) available online in convenient and compact PDF format to view and print at www.qiagen.com/safety.

CAUTION: Handle human blood as if potentially infectious.

Observe relevant blood handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

Direct draw into QFT-Plus Blood Collection Tubes

1. Label tubes appropriately. Ensure each tube (Nil, TB1, TB2, and Mitogen) is identifiable by its label or other means once the cap is removed.
2. For each patient, collect 1 ml of blood by venipuncture directly into each of the QFT-Plus Blood Collection Tubes. A trained phlebotomist should perform this procedure.

Important note: Tubes should be between 17° to 25°C at the time of blood filling.

A. As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling. This will ensure correct draw volume.

B. The black mark on the side of the tubes indicates the validated range of 0.8–1.2 ml. If the level of blood in any tube is outside the range of the indicator mark, obtain a

new blood sample. Under or over-filling of the tubes outside of the 0.8 to 1.2 ml range may lead to erroneous results.

C. If using a "butterfly needle" to collect blood, use a "purge" tube to ensure that the tubing is filled with blood prior to using the QFT-Plus tubes.

D. Use QFT-Plus Blood Collection tubes up to an altitude of 810 meters (2650 feet) above sea level. Use HA QFT-Plus Blood Collection Tubes at altitudes between 1020 and 1875 meters (3350 and 6150 feet).

E. If using QFT-Plus Tubes at an altitude higher than 810 meters (2650 ft), but not between 1020 m (3350 ft) and 1875 m (1610 ft), or if low blood-draw volume occurs, users can collect blood with a syringe and immediately transfer 1 ml of blood to each of the 4 QFT-Plus tubes. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus tubes, and adding 1 ml of blood to each tube (to the center of the black mark on the side of the tube label). Ensure each tube (Nil, TB1, TB2, and Mitogen) is identifiable by its label or other means once the cap is removed. Replace the caps securely and mix as described below. Alternatively, blood may be collected in a single generic blood collection tube containing lithium heparin or sodium heparin as the anticoagulant and then transferred to the QFT-Plus tubes. Only use lithium heparin or sodium heparin as a blood anticoagulant because other anticoagulants interfere with the assay. Fill a blood collection tube (5-ml minimum volume) and gently mix by inverting the tube several times to dissolve the lithium heparin or sodium heparin. Blood tubes must be maintained and transported at room temperature (22°C ± 5°C) before transfer to QFT-Plus tubes for incubation, which must be initiated within 16 hours of blood collection. If blood has been collected in a lithium-heparin or sodium-heparin tube, samples must be evenly mixed by gentle inversion before dispensing into QFT-Plus tubes. Perform dispensing aseptically (ensuring appropriate safety procedures) by removing the caps from the 4 QFT-Plus tubes and adding 1 ml of blood to each (to the center of the black mark on the side of the tube label). Replace the tube caps securely and mix as described below.

3. Immediately after filling tubes, shake them ten (10) times just firmly enough to ensure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.

   Important note: Tubes should be between 17° to 25°C at the time of shaking. Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

4. Following labeling, filling, and shaking, the tubes must be transferred to a 37°C ± 1°C incubator as soon as possible and within 16 hours of collection. Prior to incubation, maintain and transport the tubes at room temperature (22°C ± 5°C).

   If the blood is not incubated immediately after collection, users must immediately re-mix the tubes by inverting 10 times prior to incubation.

5. Incubate the tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO_2 or humidification.

Blood Collection into a single lithium- or sodium-heparin tube and then transfer to QFT-Plus Blood Collection Tubes

1. Blood may be collected in a single blood collection tube containing lithium or sodium heparin as the anticoagulant and then transferred to QFT-Plus Blood Collection Tubes. Only use lithium or sodium heparin as a blood anticoagulant because other anticoagulants interfere with the assay. Label tubes appropriately.

   It is recommended to label the tube with the time and date of the blood collection.

   Important: Blood collection tubes should be at room temperature (17–25°C) at the time of blood collection.

2. Fill a lithium- or sodium-heparin blood collection tube (minimum volume 5 ml) and gently mix by inverting the tube several times to dissolve the heparin. This procedure should be performed by a trained phlebotomist.

3. Hold time and temperature options for lithium- or sodium-heparin tubes prior to transfer and incubation in QFT-Plus Blood Collection Tubes (See Figures 1–3 Blood Collection Options).

   Option 1 – Lithium- or Sodium-Heparin Tube Room Temperature Storage and Handling

   Blood collected in lithium- or sodium-heparin tube must be maintained at room temperature (22°C ± 5°C) for no more than 16 hours from the time of collection prior to transfer to QFT Plus Blood Collection Tubes and subsequent incubation.

   Option 2 – Lithium- or Sodium-Heparin Tube Refrigerated Storage and Handling

   Important: Procedural steps a–d must be followed in sequence.

   a. Blood drawn into lithium- or sodium-heparin tube may be held at room temperature (17–25°C) up to 3 hours after blood collection.
   b. Blood drawn into lithium- or sodium-heparin tube may be refrigerated (2–8°C) for up to 48 hours.
   c. After refrigeration, lithium- or sodium-heparin tube must equilibrate to room temperature (17–25°C) prior to transfer to QFT-Plus Blood Collection Tubes.
   d. Aliquoted QFT-Plus Blood Collection Tubes should be placed in the 37°C incubator within 2 hours of blood transfer.

   If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after transfer to QFT-Plus Blood Collection Tubes and shaking, invert the tubes to mix 10 times prior to incubation at 37°C. Total time from blood draw to incubation in QFT-Plus Blood Collection Tubes should not exceed 53 hours.

4. Transfer of blood specimen from a lithium- or sodium-heparin tube to QFT-Plus Blood Collection Tubes (Important: QFT-Plus Blood Collection Tubes should be at room temperature [17–25°C (62.6 –77°F)] at the time of blood collection.):

   a. Label each QFT-Plus Blood Collection Tube appropriately.

      Ensure each tube (Nil, TB1, TB2, and Mitogen) is identifiable by its label or other means once the cap is removed. It is recommended to transfer the recorded time

and date of blood collection from the lithium- or sodium-heparin tubes to the QFT-Plus Blood Collection Tubes.

b. Samples must be evenly mixed by gentle inversion before dispensing into QFT Plus Blood Collection Tubes.

c. Dispensing should be performed aseptically, ensuring appropriate safety procedures, removing the caps from the 4 QFT-Plus Blood Collection Tubes and adding 1 ml of blood to each tube. Replace the tube caps securely and mix as described below. Ensure each tube (Nil, TB1, TB2 and Mitogen) is identifiable by its label or other means once the cap is removed.

5. Mix tubes. Immediately after filling the QFT-Plus Blood Collection Tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls.

   Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

6. Following labeling, filling and shaking, the tubes must be transferred to a 37°C ± 1°C incubator within 2 hours. If QFT-Plus Blood Collection Tubes are not incubated at 37°C directly after blood collection and shaking, invert the tubes to mix 10 times (10x) prior to incubation at 37°C. (See Figures 1–3, next page, for blood collection options).

7. Incubate the QFT-Plus Blood Collection Tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours.

   The incubator does not require CO_2 or humidification.

Draw into QFT Plus Blood Collection Tubes and hold at room temperature.

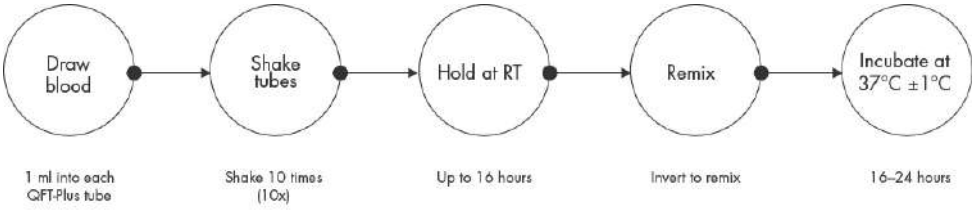


Figure 1. Blood collection option: Directly draw into QFT-Plus Blood Collection Tubes and hold at room temperature. The total time from blood draw in QFT-Plus Blood Collection Tubes to 37°C incubation must not exceed 16 hours.

Draw into lithium- or sodium-heparin tube and hold at room temperature.

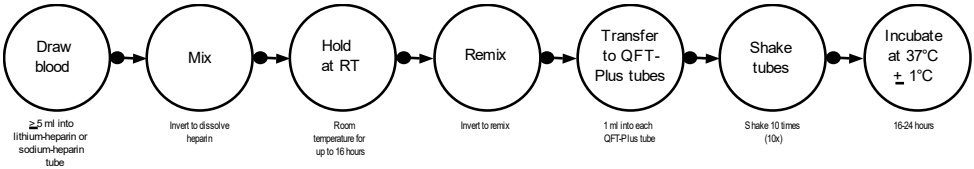
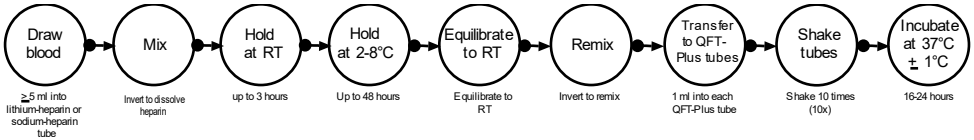


Figure 2. Blood collection option: Draw into lithium- or sodium-heparin tube and hold at room temperature.
The total time from blood draw in lithium- or sodium-heparin tube to 37°C incubation must not exceed 16 hours.

Draw into lithium- or sodium-heparin tubes and hold at 2–8°C.



Aliquotted QFT-Plus Blood Collection Tubes should be placed in a 37°C incubator within 2 hours of blood transfer to QFT-Plus Blood Collection Tubes.

Figure 3. Blood collection option: Draw into lithium- or sodium-heparin tube and hold at 2–8°C.
The total time from blood draw in lithium- or sodium-heparin tube to 37°C incubation must not exceed 53 hours.

8. After incubation, blood collection tubes may be held between 4°C to 27°C for up to 3 days prior to centrifugation.
9. After incubation, centrifuge tubes for 15 minutes at 2000 to 3000 RCF (*g*). The gel plug will separate the cells from the plasma. If this does not occur, centrifuge the tubes again.

   It is possible to harvest the plasma without centrifugation; however, this requires additional care to remove the plasma without disturbing the cells.
10. Harvest plasma samples using only a pipette.

    Important note: After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

    Plasma samples can be loaded directly from centrifuged blood collection tubes into either the QFT-Plus ELISA plate, including when automated ELISA workstations are used, or onto the LIAISON QuantiFERON-TB Gold Plus System (REF: 311010). Plasma samples can be stored for up to 28 days at 2–8°C or, if harvested, below –20°C for extended periods.

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Legal manufacturer
	CE-IVD marked symbol
	For in vitro diagnostic use
	Global Trade Item Number
	Temperature limitation
	Consult instructions for use
	Do not reuse
	Material number
Rn	R is for revision of the Instructions for Use and n is the revision number

Handbook Revision History

Document	Changes
R5 04/2019	Lithium heparin/Sodium heparin changes New work instructions for 2–8°C blood collection workflow
R6 08/2019	Added reference to QFT-Plus ELISA to front cover
R7 08/2019	Removed extra page

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www.quantiferon.com

To whom it might concern,

We, herewith confirm that the QuantiFERON-TB Gold Plus product line has a self-declared CE mark under the IVD Directive 98/79/EC.

The devices are not defined as high-risk (Annex II, List A/B) and do not require further EC certification.

Manufacturer ISO 13485 certification and EU an declaration of conformity are available upon request.

QIAGEN GmbH
QIAGEN STRASSE 1
40724
HILDEN
GERMANY

Yours Faithfully,

Samuel Hughes
Senior Specialist, Regulatory Affairs

Date: 03-Apr-2020

DE 11501 83 certified copy

EC Declaration of Conformity

Name and address of the company

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
Germany

We herewith declare under our sole responsibility that the product

QuantiFERON®-TB Gold Plus	REF No 622120 and 622822
QFT-Plus Single Patient Pack (pack of 10)	REF No 622222
QFT-Plus tubes (50x TB1/TB2/Nil/Mitogen)	REF No 622526
QFT-Plus Dispenser Pack (25ct)	REF No 622423
QFT-Plus Reference Lab Pack	REF No 622822
QFT-Plus HA Single Patient Pack (pack of 10)	REF No 623222
QFT-Plus HA Dispenser Pack (25ct)	REF No 623423
QFT-Plus HA tubes (50x TB1/TB2/Nil/Mit)	REF No 623526

Classification General IVD

Conformity Asessement Route Annex III

and meets all applicable requirements of the following European Directive:

In Vitro Diagnostic Directive (IVDD) 98/79/EC

[QIAGEN GmbH, Hilden, September 4, 2019]

Lindsey Howard
Vice President, Global Regulatory Affairs
QIAGEN

CE

This copy is identical with
a copy of the document.

Erlangen, 17. JAN. 2020

Christian Braun
Notary in Erlangen / Germany

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Land: Bundesrepublik Deutschland
   Diese öffentliche Urkunde
2. ist unterschrieben von Notar Christian Braun
3. in seiner Eigenschaft als Notar in Erlangen.
4. Sie ist versehen mit dem Siegel des Notars Christian Braun in Erlangen.

Bestätigt

5. in Nürnberg 6. am 21. Januar 2020
7. durch den Präsidenten des Landgerichts Nürnberg-Fürth
8. unter Nr. 910 a E - 311/2020
9. Siegel 10. Unterschrift
   In Vertretung

Dr. Margit Zorn
Vizepräsidentin des
Landgerichts