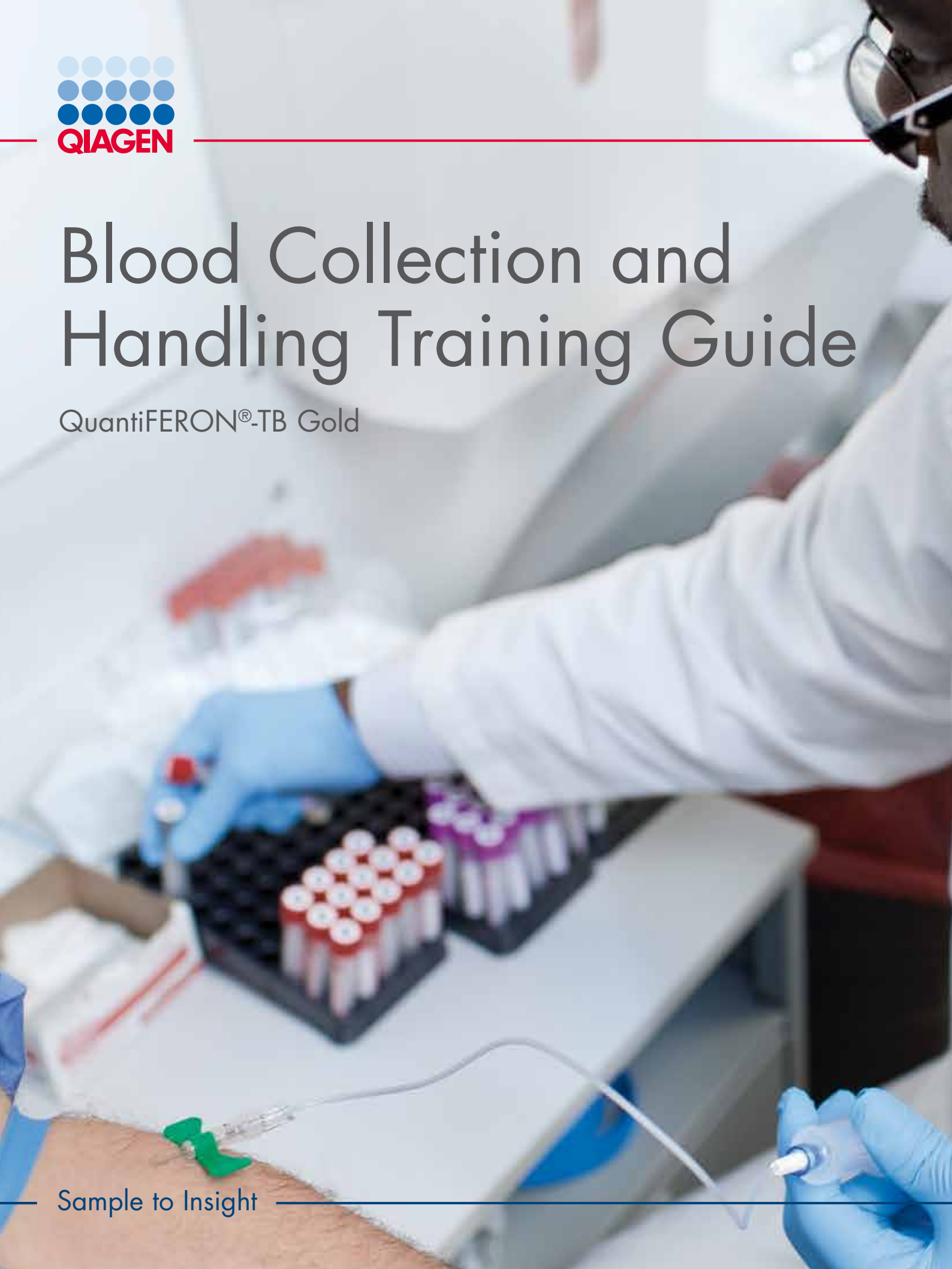
QIAGEN

Blood Collection and Handling Training Guide

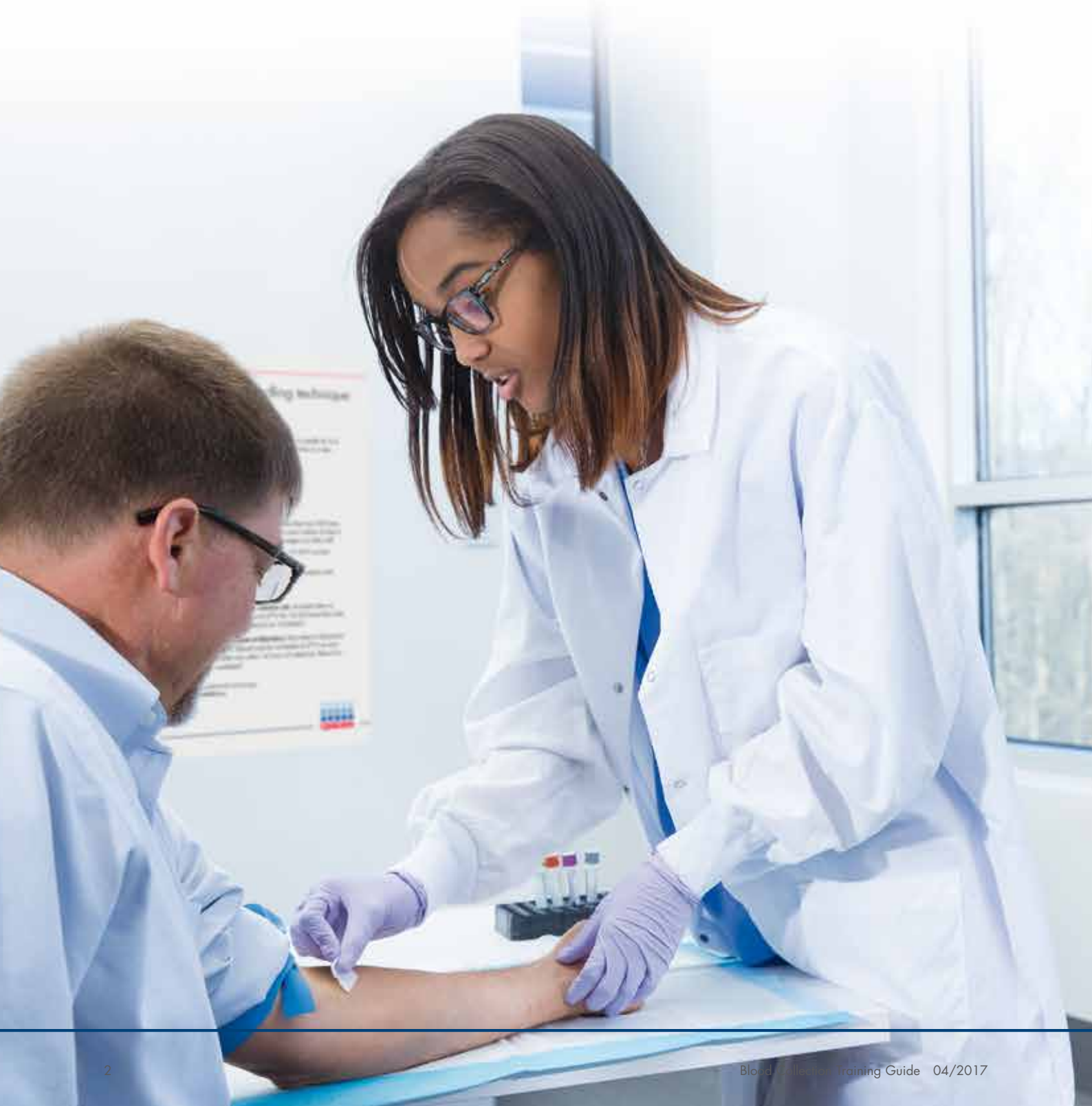
QuantiFERON®-TB Gold



Sample to Insight

Dear QuantiFERON-TB Gold User,

This guide is intended for phlebotomists and anyone involved in the initial stages of performing the QuantiFERON-TB Gold (QFT®) test and describes the stages of blood collection, incubation and shipping to the laboratory. It is to be used in conjunction with the latest version of the QFT Package Insert for your region. Visit **www.QuantiFERON.com** for the most up-to-date package insert.



QuantiFERON-TB Gold is an indirect test for *Mycobacterium tuberculosis* infection and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations. QFT is an innovative blood test that measures the cell-mediated immune response to very specific tuberculosis (TB) antigens and is commonly known as an interferon-gamma release assay (IGRA).

In this training guide, you will find:

- Information about how QFT works and compares with the tuberculin skin test (TST)
- Direction for proper QFT blood specimen collection and handling
- A link to a training video available at http://us-tb.gnowee.net/videos
- A proficiency quiz to test your knowledge after reading
- A checklist for completion and certificate of proficiency
- A poster to train your new colleagues and serve as a reminder

IGRAs are emerging as standard practice as aids to the diagnosis of latent tuberculosis infection (LTBI). In 2016, the Centers for Disease and Prevention, the American Thoracic Society and the Infectious Diseases Society of America released new guidelines for the diagnosis of tuberculosis. These guidelines recommend an IGRA, rather than a TST, for individuals who are likely to be infected with TB, are at low or intermediate risk of disease progression, and for whom it has been decided that testing for LTBI is warranted. Furthermore, an IGRA is strongly recommended for testing individuals who meet these criteria and are also vaccinated with Bacillus Calmette–Guérin (BCG) or who are unlikely to return to have their TST read. In all other situations, the use of an IGRA is an acceptable alternative to the TST, where the TST is currently being used. (1)

To make the best use of this training guide, simply follow this process:

1. Learn

a. Read through the material thoroughly.

b. Watch the video available at http://us-tb.gnowee.net/videos.

2. Apply

a. Test your understanding with the Proficiency Quiz.

b. Prove your competence by performing 5 QFT blood draws with direct observation.

3. Certify

a. Review the Certification Checklist and verify that you have completed each item.

b. Fill out a certificate and retain it for your records.

We hope this guide will effectively support your efforts in training for the collection of your patients' blood samples.

Benefits of QFT

What is QuantiFERON-TB Gold?

QFT is a laboratory test for identifying people infected with *M. tuberculosis*, the bacterium that causes TB. The test identifies all stages of infection, including infection without symptoms (LTBI) as well as infection resulting in active disease. QFT uses special blood collection tubes coated with antigens (small non-infectious bits of the TB bacterium) for blood collection and subsequent testing. These antigens are very specific for detecting TB infection. When the blood of an individual infected with TB comes into contact with these antigens, a chemical messenger called interferon-gamma (IFN-γ) is released into the blood. An enzyme-linked immunosorbent assay (ELISA) laboratory test is used to detect and quantify the amount of IFN-γ that has been released.

What is the tuberculin skin test?

Until recently, the 110-year old TST was the only method widely used to identify patients with LTBI. The TST is performed by injecting purified tuberculin protein derivative intradermally in the inner surface of the forearm. If the person has reactivity to the tuberculin, they will develop redness and swelling (induration) at the injection site. Individuals need to return to the clinic between 48 and 72 hours after administration to have any induration measured. If the diameter of induration is above the test's cut off, the person will be deemed positive. A positive response can indicate active TB or LTBI, but may also be due to prior BCG vaccination, reactivity to mycobacteria that do not cause TB, or other factors.

Some of the major benefits of QFT compared with the TST

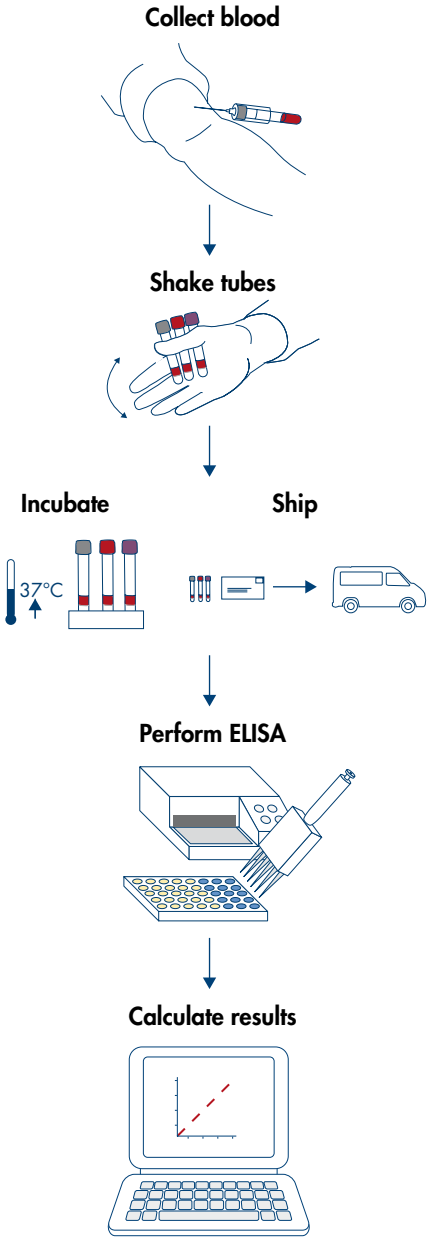
QFT	TST
QFT is performed without the need for a return visit to have results read	Patients must visit the clinic twice to complete the TST. Failure to return to have the test results read necessitates patient returning to have the test re-administered
QFT is not affected by BCG vaccination (2)	Previous vaccination with BCG can lead to false positive TST results in patients who are not infected with tuberculosis (3)
QFT is >99% specific, virtually eliminating false-positive readings	False positives by TST range from 3% to 65% of all persons tested, dependent upon the population (4)
QFT is not subject to boosting, eliminating the need for 2-step testing (5)	An initial TST may cause future TSTs to be subject to boosting, resulting in increased potential for false positives (3)

QFT is a modern alternative to the TST and offers improved performance in the following ways:

- QFT is significantly more accurate than the TST in identifying people who will progress to active TB disease (6)
- QFT is significantly more sensitive, nearly halving the number of infected people missed by the TST (3)

QFT procedure: simple, reliable and reproducible

The QFT assay is a straight-forward laboratory test that involves the following steps:



Collect 1 ml of blood into Nil, Antigen, and Mitogen tubes.

Immediately after filling, shake tubes 10 times just firmly enough to ensure that the inner surface of the tube is coated in blood (to dissolve antigens on tube walls).
Warning: Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

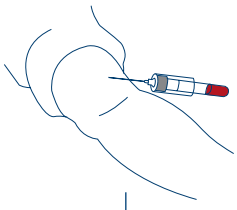
Depending on your setup, tubes can either be:
- Incubated then shipped to lab
- Sent directly to lab for incubation

Centrifuge tubes to separate plasma. Complete the ELISA procedure and obtain absorbance values.

Analyze and interpret results using QFT Analysis Software.

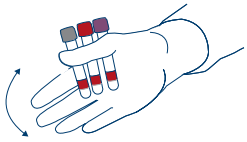
Figure is for illustrative purposes only. Full instructions for use can be found in the QFT Package Insert, available in up to 25 different languages, at **www.QuantiFERON.com**.

Technical information on blood collection, incubation and shipping



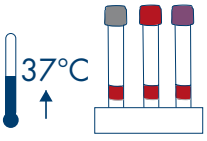
1. Blood collection

- Collect 1 ml of blood by venipuncture into each QFT blood collection tube.
- Tubes should be at 17–25°C at the time of blood filling.
- QFT blood collection tubes have been validated for volumes ranging from 0.8 ml to 1.2 ml.
- Tubes fill slowly — hold tube on needle for 2 to 3 seconds after flow ceases. If blood level is not close to the black mark on the side of the tube label, obtain another sample.
- If using a butterfly needle, prime tubing with a "purge" tube (not supplied) before filling QFT tubes.



2. Blood tube mixing

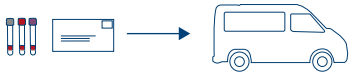
- Immediately after filling, shake tubes 10 times just firmly enough to ensure that the inner surface of the tube is coated in blood (to dissolve antigens on tube walls).
- Overly vigorous shaking may cause gel disruption and could lead to aberrant results.
- Label tubes appropriately.



3. Shipping and incubation

Option 1: Incubate at collection site

- Blood must be incubated as soon as possible and within 16 hours of collection. Incubate tubes upright at 37°C ± 1°C for 16 to 24 hours.
- If tubes are not incubated at 37°C ± 1°C soon after collection, re-mix tubes by inverting 10 times immediately prior to incubation.
- Ship incubated tubes to testing laboratory. After incubation tubes may be stored at 4–27°C for up to 3 days prior to centrifugation at the testing laboratory.
- Technical tip: Label tubes as "Incubated".



Option 2: Incubate at laboratory

- Ship tubes to laboratory at 17–27°C.
- Blood must be incubated at 37°C ± 1°C as soon as possible and within 16 hours of collection.
- Re-mix tubes by inverting 10 times immediately prior to incubation.
- Technical tip: Label tubes as "Not Incubated".

Warning: Standard blood handling precautions apply.

Technical information on tube filling, mixing and incubation

Filling tubes

One QFT test uses the following 3 collection tubes (Figure 1):

- Nil, negative control (grey cap) — adjusts for background noise
- TB Antigen (red cap) — assesses IFN-γ response to highly-specific TB antigens
- Mitogen, positive control (purple cap) — serves as an IFN-γ positive control for each specimen tested

These procedures should be followed for optimal results:

- Tubes should be at 17–25°C at the time of blood filling.
- Collect 1 ml of blood by venipuncture directly into each QFT blood collection tube.

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

- The black mark on the side of the tubes indicates the 1 ml fill volume. QFT blood collection tubes have been validated for volumes ranging from 0.8 ml to 1.2 ml. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample. As a guide, the picture on the right illustrates the approved fill range (Figure 2).

If a "butterfly needle" is used, prime tubing with a "purge" tube before filling the QFT tubes.

Figure 1. QFT collection tubes: (left to right) Nil, TB Antigen and Mitogen.

Figure 2. Approved fill volume range; image for guidance only.

Mixing

Antigens have been dried onto the inner wall of the blood collection tubes. It is essential that the tubes' contents be thoroughly mixed with the blood. Thorough mixing will dissolve the heparin, preventing clotting, and allow resolubilization of the stimulating antigen. Mixing is performed by shaking the tubes 10 times just firmly enough to ensure that the entire inner surface of the tube is coated with blood. Firm inversion is not an acceptable mixing method. Frothing of blood in the QFT blood collection tubes after the shaking process is normal (Figure 3).

Incubation

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 tubes at room temperature (22°C ± 5°C). Do not refrigerate or freeze the blood samples. Tubes should be incubated upright. Following 37°C ± 1°C incubation, blood collection tubes may be held between 4°C and 27°C for up to 3 days prior to centrifugation.

If the blood is not incubated immediately after collection, the tubes must be mixed by inverting 10 times immediately prior to incubation.

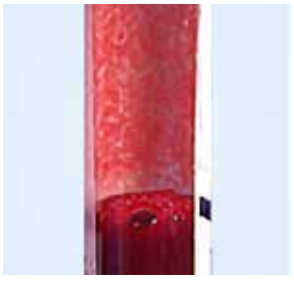


Figure 3. A correctly mixed tube.

Proficiency quiz

The objective of this proficiency quiz is to ensure each participant fully understands the specimen collection and handling process for the QuantiFERON-TB Gold test. Prior to completing this quiz you must have read the materials thoroughly and have watched the video available on http://us-tb.gnowee.net/videos. The minimum score to demonstrate proficiency is 80%.

1. How does testing for TB with QFT differ from the TST? Choose the most appropriate answer:

a. TST is an in vitro test; QFT is an intradermal test

b. TST is an intradermal test that measures a person's immune response to non-specific *Mycobacterium* proteins, whereas QFT is an in vitro test which measures a person's immune response to proteins found in *M. tuberculosis*, but not in the BCG vaccine and most non-tuberculosis mycobacteria

2. What does QFT measure? Choose the most appropriate answer:

a. A person's immune response to non-specific *Mycobacterium* proteins

b. A person's immune response to proteins found in *M. tuberculosis*. The IFN-γ levels indicate the level of a person's immune response

3. Blood can be collected into which of the following collection tubes for QFT testing? Please choose the correct answer:

a. Blue top tube

b. Red top, silicon tube

c. Three unique QFT blood collection tubes: Nil (grey top), TB Antigen (red top) and Mitogen (purple top)

4. Choose the correct answer. The Nil tube (grey top):

a. Is a negative control; it is used to adjust for background noise

b. Serves as a back-up in case the red or purple top tubes do not perform adequately

c. Is for the detection of other respiratory infections

5. Choose the correct answer. The Mitogen tube (purple top):

a. Serves as a back-up in case the red or grey top tubes do not perform adequately

b. Is used as a positive control for correct tube handling and for the production of any IFN-γ

6. Choose the correct answer. The TB Antigen tube (red top):

a. Is coated with TB-specific antigens, which should result in IFN-γ production by white blood cells from individuals with TB infection

b. Serves as a positive control for the production of any IFN-γ

c. Detects other mycobacteria (non-TB)

7. At what temperature should the QFT blood collection tubes be stored? Please choose the correct answer:

a. 4°C to 25°C

b. 4°C to 40°C

c. Room temperature (defined in QFT Package Insert as 17°C to 27°C)

8. Please choose the correct answer. At the time of blood collection, tube temperature should be:

a. Room temperature (defined in QFT Package Insert as 17°C to 27°C)

b. 2°C to 8°C

c. 17°C to 25°C

9. What is the acceptable fill volume for QFT blood collection tubes? Please choose the correct answer:

a. 0.8 ml to 1.2 ml

b. 1 ml precisely

c. Any volume is acceptable

10. What is the procedure for QFT blood collection tubes with volumes outside the acceptable range? Please choose the correct answer

a. Affected tube should be discarded and blood redrawn into the same type of tube

b. No change, sample is acceptable

c. Open tube and discard excess blood

11. Choose the appropriate phrase to complete the following sentence: When a "butterfly needle" is being used to collect blood, which is often done when many tubes require blood collection, …

a. … the procedure is identical as using regular needle

b. … a volume lower than 1 ml is acceptable because butterfly needles are used mostly with children

c. …a "purge" tube should be used to ensure that the tubing is filled with blood prior to the QFT tubes being filled

12. After blood is collected into the QFT blood collection tubes, what is the next step in the process? Please choose the correct answer:

a. Incubation of tubes

b. Courier to laboratory for processing and analyzing

c. Shaking tubes 10 times, just firmly enough to ensure the entire inner surface of the tube is coated with blood

13. What is the purpose of shaking (i.e., versus inverting) the QFT blood collection tubes? Please choose the correct answer:

a. To solubilize the antigens on the tube walls, initiating the immunological reaction

b. To ensure white blood cells are evenly distributed throughout the blood

14. Is firm inversion an acceptable mixing method immediately after blood collection into the QFT tubes? Please choose the correct answer:

a. No

b. Yes

15. Is frothing of blood in the QFT blood collection tubes normal after the shaking process? Please choose the correct answer:

a. No

b. Yes

16. After blood collection and shaking of the filled QFT blood collection tubes, what are the options for sample handling prior to incubation? Please choose the correct answer:

a. Within 16 hours of collection and shaking, QFT samples must be incubated at 37°C ± 1°C for 16 to 24 hours. QFT samples should be maintained at 22°C ± 5°C during transportation to the lab for incubation

b. QFT samples should be refrigerated and transported to the lab for incubation at 37°C ± 1°C

17. Choose the appropriate phrase to complete the following sentence: If tubes are not incubated at 37°C ± 1°C immediately after filling, they should — within 16 hours of blood collection — be …

a. … placed in a 37°C ± 1°C incubator standing upright for 16 to 24 hours

b. … re-mixed by inverting ten times prior to placing upright in an incubator at 37°C ± 1°C for 16 to 24 hours

Proficiency quiz answers

1 b. 2 b. 3 c. 4 a. 5 b. 6 a. 7 a. 8 c. 9 a. 10 a. 11 c. 12 c. 13 a. 14 a. 15 b. 16 a. 17 b.

For comprehensive instructions for use, please refer to the Package Insert, available in up to 25 different languages, on **www.QuantiFERON.com**.

References

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QFT is approved by the US FDA

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. FDA approval notes that 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. QFT Package Inserts, available in up to 25 different languages, can be found at **www.QuantiFERON.com**.

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