

FAQs for Laboratory Professionals

QuantiFERON®-TB Gold Plus



Contents

| | |
|---|---|
| Questions and answers | 3 |
| Test principle | 3 |
| Blood collection | 4 |
| The blood hasn't reached the black mark on the side of the QFT-Plus blood collection tube. Is this important? | 4 |
| How important is the tube mixing process? | 4 |
| Can the blood collection tubes be transported lying down? | 4 |
| Blood incubation / plasma harvesting | 4 |
| What if 37°C incubation starts more than 16 hours after the time of blood collection for specimens collected directly into QFT-Plus blood collection tubes? | 4 |
| Can I incubate the blood collection tubes lying down? | 5 |
| Do I have to centrifuge the tubes before I can harvest the plasma? | 5 |
| Do I have to centrifuge the tubes immediately after removal from the incubator? | 5 |
| The gel plug hasn't moved during centrifugation. What should I do? | 5 |
| The plasma doesn't appear the color it normally does. Is this OKAY? | 5 |
| Do I require a Class II Biohazard Cabinet in which to perform plasma harvesting? | 6 |
| What volume of plasma do I need to harvest from above the sedimented red blood cells or gel plug? Is this important? | 6 |
| I want to maximize the cost-effectiveness of the QuantiFERON-TB Gold Plus assay by batching my samples. What is the stability associated with the harvested plasma? | 6 |
| What should I do if clots form in my plasma samples during frozen storage? | 6 |
| Do I need to use microtubes when storing harvested plasma? | 6 |
| Can I use more cost-effective microtiter plates in this instance? | 6 |
| Interferon-gamma (IFN- γ) ELISA | 7 |
| What is the stability associated with these selected kit components— | 7 |
| Can I use the QuantiFERON ELISA plate immediately after removal from the refrigerator? | 7 |
| Do I require an automated Plate Washer? | 7 |
| How important is washing during the QuantiFERON ELISA? | 7 |

| | |
|--|---|
| Data analysis | 8 |
| I have very high Nil control values? What may be the problem? | 8 |
| A patient's TB Antigen value is very high (possibly above the detectable limit of the plate reader). Is this OKAY? | 8 |
| Can the amount of IFN- γ measured be correlated to the stage or degree of TB infection? | 8 |
| Is there an easy way of calculating and interpreting QuantiFERON-TB Gold Plus test results? | 8 |
| Troubleshooting | 9 |

Questions and answers

These Frequently Asked Questions (FAQs) relate to the QuantiFERON-TB Gold Plus (QFT®-Plus) assay. The answers provided are meant to act as a guide only. We recommend that the QuantiFERON-TB Gold Plus Package Insert be used as the reference for test procedures, as well as for all other enquiries relating to the use or performance of the assay.

Test principle

The QuantiFERON-TB Gold Plus assay is an in vitro diagnostic laboratory test that aids in the indirect detection of infection with *Mycobacterium tuberculosis*. It uses human whole blood, with patented assay technology based on the measurement of Interferon-gamma (IFN- γ) secreted from stimulated T-cells previously exposed to *M. tuberculosis*.

The QFT-Plus assay is a straightforward laboratory test that involves the following steps:

- Collection or transfer of blood into QFT-Plus blood collection tubes.
- Incubation at 37°C.
- Detection of released IFN- γ in harvested plasma using an ELISA.
- Analysis and results using the QFT-Plus Analysis Software.

Blood collection

The blood hasn't reached the black mark on the side of the QFT-Plus blood collection tube. Is this important?

The black mark on the side of the tubes indicates the range of blood that QFT-Plus blood collection tubes have been validated for; volumes ranging from 0.8 to 1.2 ml. If the level of blood in any tube is not within the indicator mark, another blood sample should be obtained.

How important is the tube mixing process?

The antigen mixing process ensures even distribution of stimulating antigens to allow antigen processing and stimulation of IFN- γ secretion. It is a very important step in the QFT-Plus assay—poor mixing may lead to erroneous results.

Immediately after filling the tubes, shake them ten (10) times just firmly enough to ensure that the entire inner surface of the tube has been coated with blood, to solubilize antigens on the tube wall. Thorough mixing is required to ensure proper mixing of the blood with the tube's contents. Some blood frothing is expected and will not adversely affect the performance of the test. Universal blood handling precautions should be used. Tubes should be between 17–25°C at the time of blood filling.

Technical notes on blood handling procedures are available at www.QuantiFERON.com

Can the blood collection tubes be transported lying down?

Yes. QFT-Plus blood collection tubes can be transported lying down after the tube shaking has been performed. The tubes should be mixed again by inverting 10 times immediately prior to being placed upright in the 37°C \pm 1°C incubator.

Alternatively, blood collected in lithium-heparin tubes should be maintained at room temperature (22°C \pm 5°C) prior to transfer to the QFT-Plus blood collection tubes.

Blood incubation / plasma harvesting

What if 37°C incubation starts more than 16 hours after the time of blood collection for specimens collected directly into QFT-Plus blood collection tubes?

Blood specimens collected directly into QFT-Plus blood collection tubes and incubated more than 16 hours after collection are likely to exhibit a decreased IFN- γ response due to cellular breakdown (death), leading to loss of sensitivity and inaccurate results.

Blood specimens can also be collected in lithium-heparin tubes. Blood in lithium-heparin tubes should be maintained and transported at room temperature (22°C \pm 5°C) before transfer to QFT-Plus tubes for incubation, which must be initiated within 16 hours of blood collection.

Can I incubate the blood collection tubes lying down?

QFT-Plus blood collection tubes must be kept upright during incubation at 37°C.

Do I have to centrifuge the tubes before I can harvest the plasma?

While it is recommended to centrifuge the tubes to assist with harvesting, it is possible to harvest the plasma from the tubes without centrifugation. However, additional care is required to remove the plasma without disturbing the cells.

Do I have to centrifuge the tubes immediately after removal from the incubator?

After incubation, QFT-Plus blood collection tubes may be held between 4°C and 27°C for up to 3 days before centrifugation or harvesting.

The gel plug hasn't moved during centrifugation. What should I do?

After incubation of tubes at 37°C, the plasma is separated from the cells by centrifuging for 15 minutes at 2000–3000 RCF (g). The gel plug should move to separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged.

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 should only be harvested using a pipette.
- Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT-Plus ELISA plate.
- Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below –20°C (preferably ≤–70°C) for extended periods.

The plasma doesn't appear the color it normally does. Is this OKAY?

Plasma from the QFT-Plus blood collection tubes can appear more red than usual—this is normal. It should be noted that the color of plasma, even those without any red blood cell contamination, can vary from almost colorless to shades of yellow/pale brown; some plasma samples even have an opaque appearance. These qualities have been found not to affect QFT-Plus results.

Do I require a Class II Biohazard Cabinet in which to perform plasma harvesting?

Ideally, all work with blood should be performed in a Biohazard Cabinet to minimize the risk of infection (e.g. HIV, Hepatitis-B) from potentially infectious blood samples. However, if aseptic techniques are used, plasma harvesting can be performed outside of a Biohazard cabinet. 'Safe Laboratory Practices' should be followed, including the use of protective clothing such as gloves, gown, safety eyewear, etc. as suggested by relevant local requirements.

What volume of plasma do I need to harvest from above the sedimented red blood cells or gel plug? Is this important?

As little as 150 μ l of plasma is sufficient, as only 50 μ l of plasma is required to perform the ELISA. Two-hundred μ l will leave sufficient plasma for reference (re-testing) purposes, if required. It is generally possible to remove greater than 300 μ l. Validation studies have shown that IFN- γ is evenly distributed in the plasma and the volume removed is not critical. The volume of plasma available can vary from patient to patient.

Note: After centrifugation and prior to harvesting, avoid pipetting up and down or mixing plasma.

I want to maximize the cost-effectiveness of the QuantiFERON-TB Gold Plus assay by batching my samples. What is the stability associated with the harvested plasma?

Harvested plasma (or plasma stored in the blood collection tubes after centrifugation) can be stored at 2–8°C for up to 28 days, or below –20°C (preferably \leq –70°C) for extended periods. Plasma kept at \leq –70°C are less likely to form fibrin clots.

What should I do if clots form in my plasma samples during frozen storage?

Upon thawing, frozen plasma samples may require centrifugation to sediment the fibrin clots that can form during storage. A guide to dealing with clotted plasma samples is outlined in the Package Insert.

Do I need to use microtubes when storing harvested plasma? Can I use more cost-effective microtiter plates in this instance?

Uncoated low-binding microtiter plates, with an appropriate sealed covering to prevent evaporation, can be used to store harvested plasma.

Interferon-gamma (IFN- γ) ELISA

What is the stability associated with these selected kit components—

a) Kit Standard?

Reconstituted IFN- γ kit standard may be kept for up to 3 months if stored at 2–8°C (the date of reconstitution should be noted). Reconstituted kit standard should be equilibrated to room temperature (17–27°C) for 1 hour before use.

b) Conjugate 100X Concentrate?

Once reconstituted, the Conjugate 100X Concentrate must be used within 3 months or discarded. Working strength conjugate (Conjugate 100X Concentrate mixed with Green Diluent) must be used within 6 hours of its preparation. Any unused Conjugate 100X Concentrate must be returned immediately to 2–8°C following its use.

c) Wash Buffer?

Working strength Wash Buffer may be stored at room temperature (17–27°C) for up to 2 weeks.

Can I use the QuantiFERON ELISA plate immediately after removal from the refrigerator?

No. Sealed ELISA plates should be allowed to equilibrate to room temperature (17–27°C) for at least 1 hour before opening the foil bag.

Do I require an automated Plate Washer?

No. Although an automated plate washer is recommended, manual washing can be performed following the procedure as outlined in the Package Insert.

How important is washing during the QuantiFERON ELISA?

As with most ELISAs, inadequate or incorrect washing is one of the most common causes of QuantiFERON ELISA error. If you have any such problems, please check the following—

- If bubbles and froth form during the wash steps, the flow rate of the wash cycle should be adjusted (usually lowered) to prevent this from occurring.
- Wash volumes should allow the wash buffer to reach the top of each well (preferably with a positive meniscus forming over the rim of each well).
- Ensure all wells receive sufficient and equal wash buffer. Blocked washer probes can be cleaned according to the manufacturer's instructions.
- Six complete washes are suggested as a minimum in the Package Insert, however additional washes can be performed without affecting the performance of the assay.
- A soak period of at least 5 seconds between each cycle is recommended.

Data analysis

I have very high Nil control values? What may be the problem?

Under most circumstances, the expected IFN- γ concentration range for the Nil control is below 1 IU/ml (however, values up to 8 IU/ml can be acceptable). If Nil control values are greater than this or are occurring at a high frequency, the result may be due to a technical error. In such instances judgement should be used in determining whether to re-test.

A patient's TB Antigen value is very high (possibly above the detectable limit of the plate reader). Is this OKAY?

In some cases the patient's TB Antigen IFN- γ level may be above the limit of the microplate reader—such an occurrence will have no impact on the test interpretation, provided the result for that patient's Nil control is below 8 IU/ml.

Can the amount of IFN- γ measured be correlated to the stage or degree of TB infection?

No. Individuals displaying a response greater than or equal to 0.35 IU/ml above the Nil control (and greater than or equal to 25% of the Nil value), for the TB Antigen tube/s, are likely to be infected with *M. tuberculosis*. No correlation between their response to these antigens and the stage or degree of infection, their level of immune responsiveness, or their likelihood for progression to active disease can be made, based on currently available data.

Is there an easy way of calculating and interpreting QuantiFERON-TB Gold Plus test results?

An outline of the Data Analysis and Test Interpretation method for the QFT-Plus assay is provided in the Package Insert. Calculation of QFT-Plus results can be performed using a spreadsheet program.

Alternatively, QFT-Plus Analysis Software is available from www.QuantiFERON.com to analyze raw data from the assay and calculate test results.

The QFT-Plus Analysis Software allows the simple transfer of raw data (ODs) from microplate reader software (or from any spreadsheet program).

The software performs—

- Calculation of a Standard Curve.
- Quality Control check of the standard replicates and curve.
- Calculation of all sample IFN- γ concentrations (IU/ml) from the Standard Curve.
- Reporting of a QuantiFERON result for each patient, according to the 'Interpretation of Results' guidelines outlined in the QuantiFERON-TB Gold Plus Package Insert.
- Please ensure that you are using the most current QuantiFERON-TB Gold Plus software for your region.

Troubleshooting

My results are not as I had anticipated. What could be the problem?

General ELISA problems—with the possible causes and suggested solutions—are listed in the following tables.

Nonspecific color development

| Possible cause | Comments and suggestions |
|--|--|
| 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. |
| Cross-contamination of ELISA wells | Take care while pipetting and mixing sample to minimize risk. |
| Kit/components have expired | Ensure kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date. |
| Enzyme Substrate Solution is contaminated | Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used. |
| Mixing of plasma in in QFT-Plus Blood Collection 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 | Comments and suggestions |
|------------------------------------|---|
| Standard dilution error | Ensure dilutions of the Kit Standard are prepared correctly as per this Package Insert. |
| Pipetting error | Ensure pipets are calibrated and used according to manufacturer's instructions. |
| Incubation temperature too low | Incubation of the ELISA should be performed at room temperature (22°C ± 5°C). |
| Incubation time too short | Incubation of the plate with the conjugate, standards and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution should be incubated on the plate for 30 minutes. |
| Incorrect plate reader filter used | Plate should be read at 450 nm with a reference filter of between 620 and 650 nm. |
| 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 1 hour. |
| 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 | Comments and suggestions |
|---|---|
| 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. A soak time of at least 5 seconds between cycles should be used. |
| Incubation temperature too high | Incubation of the ELISA should be performed at room temperature (22°C ± 5°C). |
| Kit/components have expired | Ensure that the kit is used within the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date. |
| 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 | Comments and suggestions |
|---|---|
| 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. A soak time of at least 5 seconds between cycles should be used. |
| Standard dilution error | Ensure dilutions of the standard are prepared correctly as per this Package Insert. |
| Poor mixing | Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate. |
| Inconsistent pipetting technique or interruption during assay setup | Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay. |

QuantiFERON-TB Gold Plus (QFT-Plus) is an in vitro diagnostic aid for detection of *Mycobacterium tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations. QFT-Plus results alone cannot distinguish active TB disease from latent infection.

QFT-Plus Package Inserts, available in multiple languages, as well as up-to-date licensing information and product-specific disclaimers can be found at www.QuantiFERON.com.

Trademarks: QIAGEN®, Sample to Insight®, QuantiFERON®, QFT® (QIAGEN Group)

PROM-11447-001 09/2017 1108401 © 2017 QIAGEN, all rights reserved.

Ordering www.qiagen.com/shop | Technical Support support.qiagen.com | Website www.QuantiFERON.com