

FAQs for Laboratory Professionals

QuantiFERON®-TB Gold Plus



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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. Refer to your QuantiFERON-TB Gold Plus Package Insert for approved test procedures, as well as for all other enquiries relating to the use or performance of the assay. Up-to-date QFT-Plus Package Inserts are available at www.QuantiFERON.com.

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.

Additional resources describing 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.

Blood collected in lithium-heparin tubes can also be transported laying down.

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 must be incubated in QFT-Plus blood collection tubes within 16 hours of blood collection. Blood samples 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.

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.

Blood specimens can be collected in lithium-heparin tubes can be stored up to 12 hours at room temperature (17–25°C) followed by transfer to the QFT-Plus tubes.

In addition, blood specimens may be collected in lithium-heparin tubes and stored for 15 minutes to 3 hours at room temperature (17–25°C) and then stored at 2–8°C for 16 to 48 hours prior to transfer to the QFT-Plus blood collection tubes.

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?

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

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 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.

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 µl of plasma is sufficient, as only 50 µl of plasma is required to perform the ELISA. Two-hundred µl will leave sufficient plasma for reference (re-testing) purposes, if required. It is generally possible to remove greater than 300 µl. 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?

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

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

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.

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 when stored at 2–8°C 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. ELISA plates should be allowed to equilibrate at room temperature (17–27°C) for at least one hour before use.

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.
- 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 period of at least 5 seconds between each cycle should be used.

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 are 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. 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. Physicians may choose to redraw a specimen or perform other procedures as appropriate.

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.

Can Biotin interfere with the QFT-Plus test result?

Biotin, also known as vitamin B7, is a water-soluble vitamin often found in multi-vitamins, prenatal vitamins, and dietary supplements marketed for hair, skin, and nail growth. Biotin interference has been reported in laboratory tests that use a biotin technology in their design. The QuantiFERON ELISA does not use biotin, or biotin technology, and therefore is not subject to this type of interference.

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.

QFT-Plus is an in vitro diagnostic aid for detection of *Mycobacterium tuberculosis* infection. It uses a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of interferon- γ (IFN- γ) by ELISA is used to identify in vitro responses to these 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.

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.

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