QFT®
QuantiFERON-TB Gold

Frequently Asked Questions

Laboratory Professionals

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These Frequently Asked Questions (FAQs) relate to the QuantiFERON®-TB Gold (QFT®) assay. The answers provided are meant to act as a guide only. We recommend that the QuantiFERON-TB Gold 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 assay is an *in vitro* diagnostic laboratory test that aids in the 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 assay is a straightforward laboratory test that involves the following steps:

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

Blood Collection

The blood hasn't reached the black mark on the side of the QFT blood collection tube. Is this important?
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 to 1.2 mL. If the level of blood in any tube is not close to the indicator mark, it is recommended to obtain another blood sample.

How important is the tube mixing process?
The antigen mixing process ensures even distribution of stimulating antigens to allow white blood cells to ingest and process antigen for presentation to T-cells, thus leading to IFN-γ secretion. It is a very important step in the QFT 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—25ºC at the time of blood filling.

Technical notes on blood handling procedures are available on www.cellestis.com.

Can the blood collection tubes be transported lying down?
Yes. QFT blood collection tubes can be transported lying down, but only 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 37ºC ± 1ºC incubator.

At what temperature can the blood be transported to another site, or held prior to incubation at 37ºC?
Blood should be held and transported at room temperature (17—27ºC). Do not refrigerate the blood or place on ice.

Blood Incubation / Plasma Harvesting

What if 37ºC incubation starts more than 16 hours after the time of blood collection?
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 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.

Can I incubate the blood collection tubes lying down?
QFT blood collection tubes must be kept upright during incubation at 37ºC.

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 blood collection tubes may be held between 4ºC and 27ºC 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 37ºC, 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 at a higher speed.

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 ELISA plate, including when automated ELISA workstations are used.
- 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 colour it normally does. Is this OK?
Plasma from the QFT blood collection tubes can appear more red than usual—this is normal. It should be noted that the colour of plasma, even those without any red blood cell contamination, can vary from almost colourless to shades of yellow/pale brown; some plasma samples even have an opaque appearance. These qualities have been found not to affect QFT results.

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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 (eg HIV, Hepatitis-B) from potentially infectious blood samples. However, as long as 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 regulators.

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 100 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 take greater than 300 µL. 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.

The QuantiFERON method also allows for direct sampling of plasma from the blood collection tubes using an automated system. This allows the plasma harvesting and ELISA to be performed with minimal operator input.
Note: After centrifugation and prior to harvesting, avoid pipetting up and down or mixing plasma by any means, manual or automated.

I want to maximize the cost-effectiveness of the QuantiFERON-TB Gold 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 2–8ºC for up to 28 days, or below -20ºC for extended periods. Plasma kept at -70ºC are less likely to form clots. For short-term storage (less than 28 days), it is better to refrigerate plasma samples rather than freeze them, due to possible fibrin clot formation.

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 clots that can form during the freeze/thaw process. 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 microtitre plates in this instance?
Uncoated low-binding microtitre plates, with an appropriate adhesive covering to prevent evaporation, can be used to store harvested plasma.

Interferon-gamma (IFN-γ) ELISA

What is the stability associated with—

a) **Kit Standard?**
Reconstituted IFN-γ kit standard may be kept for up to 3 months if stored at 2–8ºC (the date of reconstitution should be noted). Reconstituted kit standard should be equilibrated at Room Temperature (17–27ºC) 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 2–8ºC following its use.

c) **Wash Buffer?**
Working strength Wash Buffer may be stored at room temperature (17–27ºC) for up to 2 weeks.

Can I use the QuantiFERON ELISA plates immediately after their removal from the fridge?
No. Sealed ELISA plates should be allowed to equilibrate at Room Temperature 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 the single most common cause 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 5 IU/mL (however, values up to 8 IU/mL can be acceptable). If Nil control values are much greater than this, the result may be due to a technical error. In such instances judgement should be used in determining whether to re-test. If re-testing is required, it is recommended that all of the subject's plasma samples be re-assayed. If the Nil result remains high—and contamination of the sample plasma is unlikely—the Nil result is valid. A good practice is to check all Nil control values following each test to make sure they fall within, or are close to, the expected range.

A patient's TB Antigen value is very high (possibly above the detectable limit of the plate reader). Is this OK?
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 Antigen 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, 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 test results?
An outline of the Data Analysis and Test Interpretation method for the QuantiFERON assay is provided in the Package Insert. Calculation of QuantiFERON results can be performed using a spreadsheet program.

Alternatively, QuantiFERON Analysis Software is available from www.cellestis.com to analyse assay raw data, and calculate QFT results.

The QuantiFERON Analysis Software allows the simple transfer of raw data (ODs) directly 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 diagnostic result for each patient, according to the 'Interpretation of Results' guidelines outlined in the QuantiFERON-TB Gold Package Insert.
- Please ensure that you are using the most current QuantiFERON-TB Gold 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 appropriate solutions—are listed in the following tables.

Low Absorbance

Possible Cause	Solution
Standard dilution error	Ensure that dilutions of the Kit Standard are prepared correctly as per Package Insert.
Pipetting error	Ensure that pipettes deliver correct volume.
Wash Buffer dilution error	Ensure that a 1-in-20 dilution of the wash buffer concentrate is performed to prepare the working strength wash buffer.
Incubation temperature too low	Incubation of the ELISA should be performed at room temperature, 17–27°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 is 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 at least one hour.
Kit/Components have expired	Ensure kit is used within the expiry date. Ensure that reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.

Non-specific Colour Development

Possible Cause	Solution
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 when pipetting and mixing samples to minimize risk.
Components have expired	Ensure that the kit is used within the expiry date. Ensure that reconstituted Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
Components are contaminated	Avoid contamination of components.
Mixing of plasma in centrifuge 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. • Plasma samples should only be harvested using a pipette. • Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used. • Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below –20°C for extended periods

High Background

Possible Cause	Solution
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
Conjugate reconstitution/dilution error	Conjugate 100X Concentrate should be reconstituted with 300µL of distilled water. Working strength conjugate is prepared by diluting the Conjugate 100X Concentrate 1/100 in Kit Green Diluent as per the Package Insert.
Incubation temperature too high	Incubation of the ELISA should be performed at room temperature, 17–27°C.
Components have expired	Ensure that reconstituted Kit Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
Enzyme Substrate is contaminated	Discard substrate if blue colouration exists. Ensure clean reagent reservoirs are used.
Mixing of plasma in centrifuge 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. • Plasma samples should only be harvested using a pipette. • Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used. • Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below –20°C for extended periods

Poor Standard Curve

Possible Cause	Solution
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
Standard dilution error	Ensure that dilutions of the Kit Standard are prepared correctly as per Package Insert.

Standards Duplicate Variability

Possible Cause	Solution
Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
Incomplete washing of the plate	Wash the plate at least 6 times with 400µL/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.
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.

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.

Full instructions for use can be found in the Package Insert, available in up to 25 languages, at **www.cellestis.com**.

For more information, please contact the Cellestis office nearest you or visit **www.cellestis.com**.

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