October 2019

QuantiFERON®-CMV ELISA Package Insert

Σ 2 x 96

The whole blood IFN-γ test measuring responses to Human Cytomegalovirus peptide antigens

IVD For in vitro diagnostic use

CE

REF 0350-0201

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Contents

Intended Use

QuantiFERON-CMV ELISA (QF-CMV) is an in vitro assay using a peptide cocktail that simulates human cytomegalovirus (CMV) proteins to stimulate cells in heparinized whole blood. Detection of interferon-gamma (IFN-γ) by enzyme-linked immunosorbent assay (ELISA) is used to quantify in vitro responses to these peptide antigens associated with immune control of CMV infection. Loss of this immune function may be associated with development of CMV disease. The intended use of QF-CMV is to monitor a patient's level of anti-CMV immunity.

QF-CMV is not a test for determining CMV infection and should not be used to exclude CMV infection.

Summary and Explanation

CMV is a herpes virus that infects between 50–85% of adults in the population. It is a frequently occurring complication of immunosuppression, particularly after transplantation, and can significantly contribute to morbidity and mortality in transplant recipients. Current immunosuppressive therapies used to prevent the rejection of a transplanted organ have detrimental effects upon the T-lymphocytes and cell-mediated immune (CMI) responses, resulting in increased susceptibility to viral infections post-transplant. The importance of T-cell function in suppressing CMV replication is also highlighted by the fact that $CD8^+$ CMV-specific cytotoxic T-lymphocytes (CTLs) can protect against virus-associated pathogenesis. The enumeration of $CD8^+$ CMV-specific CTLs in immunosuppressed patients and the production of IFN-γ can be predictive of the risk of developing CMV disease. IFN-γ production can be a functional surrogate for the identification of CMV-specific CTLs.

QF-CMV is an assay for CMI responses to peptide antigens that simulate CMV proteins. The CMV peptides are designed to target $CD8^+$ T cells, including A1, A2, A3, A11, A23, A24, A26, B7, B8, B27, B35, B40, B41, B44, B51, B52, B57, B58, B60 and Cw6 (A30, B13) HLA Class I haplotypes covering >98% of human population. Individuals infected with CMV usually have $CD8^+$ lymphocytes in their blood that recognize these 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.

Principle of the Procedure

The QF-CMV test is performed in two stages. First, whole blood is collected into each of the QF-CMV blood collection tubes, which include a Nil Control tube, a CMV Antigen tube and a Mitogen tube.

The Mitogen tube is used in the QF-CMV test as a positive control. This may be especially warranted where there is doubt as to the individual's immune status. The Mitogen tube may also serve as a control for correct blood handling and incubation.

The tubes should be incubated at 37°C as soon as possible, and within 16 hours of collection. Following a 16–24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/ml) measured by QF-CMV ELISA.

The amount of IFN-γ in plasma samples from CMV Antigen and Mitogen tubes may often be above the upper limits of most ELISA readers, even when individuals are moderately immunosuppressed. For qualitative results, use the values calculated for neat plasma. For quantitative results, where actual IU/ml values are required, plasma samples should be diluted 1/10 in Green Diluent and assayed in the ELISA together with neat plasma.

Note: For samples that are within the range of the QF-CMV ELISA (i.e., up to 10 IU/ml), the result obtained with the neat plasma sample should be used. For such IFN-γ concentrations, values obtained using the 1/10 dilution of the plasma samples may be inexact.

A test is considered reactive for an IFN-γ response when the CMV Antigen tube reads significantly above the Nil IFN-γ IU/ml value. The Mitogen-stimulated plasma sample serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen indicates an indeterminate result when a blood sample also has a nonreactive response to the CMV 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 – such as with recent transplant patients. The Nil sample adjusts for background or nonspecific IFN-γ in blood samples. The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the CMV Antigen tube and Mitogen tube (see "Interpretation of Results" on page 24 of this Package Insert for an outline of how QF-CMV results are interpreted).

Time required to perform the assay

The time required to perform the QF-CMV assay is estimated below; the time of testing multiple samples when batched is also indicated:

37°C incubation of blood tubes:	16–24 hours
ELISA:	Approx. 3 hours for one ELISA plate
	Less than 1 hour labor
	Add 10–15 minutes for each extra plate

Materials Provided

Kit contents

Blood Collection Tubes (Single Patient Pack)	
Catalog no.	0192-0301
Number of preps	1
QuantiFERON Nil Control (gray cap)	1 tube
QuantiFERON CMV Antigen (blue cap)	1 tube
QuantiFERON Mitogen Control (purple cap)	1 tube
QF-CMV Blood Collection Tubes Package Insert	1

QuantiFERON-CMV ELISA	2-Plate Kit ELISA
Catalog no.	0350-0201
Microplate strips (12 x 8 wells) coated with murine anti-human IFN-γ monoclonal antibody	2 sets of 12 x 8 well microplate strips
Human IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal)	1 x vial (8 IU/ml when reconstituted)
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml
Conjugate 100x Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal)	1 x 0.3 ml
Wash Buffer 20x Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3',5,5' Tetramethylbenzindine)	1 x 30 ml
Enzyme Stopping Solution (contains 0.5 M H_2SO_4)*	1 x 15 ml
QF-CMV ELISA Package Insert	1

* Contains sulfuric acid. See page 9 for precautions.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- 37°C incubator; CO_2 not required
- Calibrated, variable-volume pipettes for delivery of 10–1000 µl with disposable tips
- Calibrated multichannel pipette capable of delivering 50 and 100 µl with disposable tips
- 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
- Plate lid

Warnings and Precautions

For in vitro diagnostic use

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 as if potentially infectious. Observe relevant blood handling guidelines.

The following hazards and precautionary statements apply to components of the QuantiFERON-CMV ELISA.

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: ProClin 300. Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Safety information

Further information

- Deviations from the QF-CMV 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, Human IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QF-CMV 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 are recorded.
- Discard unused reagents and biological samples in accordance with Local, State and Federal regulations.
- Do not use the QF-CMV Blood Collection Tubes or QF-CMV ELISA kits after the expiration date.
- Ensure that laboratory equipment such as plate washers and readers have been calibrated/validated for use.

Reagent Storage and Handling

Blood collection tubes

- Store QF-CMV Blood Collection Tubes at 4–25°C.
- QF-CMV Blood Collection Tubes should be between 17–25°C at time of blood filling.
- The shelf life of the QF-CMV Blood Collection Tubes is a maximum of 15 months from date of manufacture, when stored at 4–25°C.

ELISA kit reagents

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

Reconstituted and unused reagents

For instructions on how to reconstitute the reagents, please see "Stage 2: QuantiFERON-CMV ELISA for human IFN-γ" (steps 3 and 5 on pages 17 and 19).

- The reconstituted Human IFN-γ Standard may be kept for up to 3 months if stored at 2–8°C.

  Note the date on which the Human IFN-γ Standard was reconstituted.
- Once reconstituted, unused Conjugate 100x Concentrate must be returned to storage at 2–8°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 (22°C ± 5°C) for up to 2 weeks.

Specimen Collection and Handling

QF-CMV uses the following blood collection tubes:

- Nil Control (gray cap)
- CMV Antigen (blue cap)
- Mitogen Control (purple cap)

Antigens are dried onto the inner wall of the blood collection tubes, so it is essential that the contents of the tubes be thoroughly mixed with the blood. The tubes must be transferred to a 37°C incubator as soon as possible and within 16 hours of collection.

The following procedures should be followed for optimal results:

1. From each subject, collect 1 ml of blood by venipuncture directly into each of the QF-CMV Blood Collection Tubes. This procedure should be performed by a trained phlebotomist.

   QF-CMV Blood Collection Tubes can be used up to an altitude of 810 meters (2650 feet).

   If using QF-CMV Blood Collection Tubes at an altitude higher than 810 meters, or if low blood draw volume occurs, blood can be collected using a syringe, and 1 ml immediately transferred to each of the three tubes. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the caps from the three QF-CMV Blood Collection Tubes and adding 1 ml of blood to each (to the black mark on the side of the tube label). Replace the tube caps securely and mix as described below. 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, to ensure that the correct volume is drawn.

The black mark on the side of the tubes indicates the 1 ml fill volume. QF-CMV 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, a new blood sample should be obtained.

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 QF-CMV Blood Collection Tubes being used.

Alternatively, blood may be collected in a single generic blood collection tube containing lithium heparin as the anticoagulant and then transferred to QF-CMV Blood Collection Tubes. Only use lithium heparin as a blood anticoagulant since 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 heparin. This procedure should be performed by a trained phlebotomist. Blood should be maintained at room temperature (22°C ± 5°C) before transfer to QF-CMV Blood Collection Tubes for incubation, which must be initiated within 16 hours of blood collection.

2. Immediately after filling QF-CMV Blood Collection Tubes, shake the tubes 10 times just firmly enough to ensure that the entire inner surface of the tube is coated with blood, to dissolve antigens on tube walls.

   Tubes should be kept between 17°C–25°C at the time of blood filling.

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

   If blood has been collected in a lithium heparin tube, samples must be evenly mixed before dispensing into QF-CMV Blood Collection Tubes. Ensure that the blood is thoroughly mixed by gentle inversion immediately prior to dispensing. Dispense 1 ml aliquots (one per QF-CMV Blood Collection Tube) into an appropriate Nil, CMV Antigen, and Mitogen tube. This should be performed aseptically, ensuring appropriate safety procedures, removing the caps from the three QF-CMV Blood Collection Tubes and adding 1 ml of blood to each (to the black mark on the side of the tube label). Replace the tube caps securely and mix as described above.

3. Label tubes appropriately.

   Ensure each tube (Nil, CMV Antigen, Mitogen) is identifiable by its label or other means.

4. Following filling, shaking, and labeling, 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 the tubes at room temperature (22°C ± 5°C). Do not refrigerate or freeze the blood samples.

Procedure

Stage 1: Incubation of blood and harvesting of plasma

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

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

   Following incubation, blood collection tubes may be held at 4–27°C for up to 3 days prior to centrifugation.

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

   It is possible to harvest the plasma without centrifugation, but additional care is required to remove the plasma without disturbing the cells.

3. 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.

   Important: Plasma samples should only be harvested using a pipette.

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

   Plasma samples can be stored in centrifuged QF-CMV Blood Collection Tubes for up to 28 days at 2–8°C or, if harvested, below –20°C (preferably less than –70°C) for extended periods.

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

Stage 2: QuantiFERON-CMV ELISA for human IFN-γ

Refer to "Kit contents", page 8 and "Materials Required but Not Provided", page 9, for materials required to perform ELISA.

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 ELISA plate 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 one strip for the QF-CMV ELISA Standards and sufficient strips for the number of patients being tested. After use, retain frame and lid for use with remaining strips.
3. Reconstitute the Human 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 resolubilization. Reconstitution of the IFN-γ Standard to the correct volume will produce a solution with a concentration of 8.0 IU/ml.

   Note: The reconstitution volume of the Human IFN-γ Standard (kit standard) will differ between batches.

   Using the reconstituted standard, prepare a dilution series of four IFN-γ concentrations in Green Diluent (GD) (Figure 1, next page). 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 should be assayed at least in duplicate. Prepare fresh dilutions of the kit standard for each ELISA session.

Example of procedure for duplicate standards

Example of procedure for duplicate standards	
A	Label four tubes: S1, S2, S3, S4
B	Add 150 µl of GD to S1, S2, S3, S4
C	Add 150 µl of the kit standard to S1 and mix thoroughly
D	Transfer 50 µl from S1 to S2 and mix thoroughly
E	Transfer 50 µl from S2 to S3 and mix thoroughly
F	GD alone serves as the zero standard (S4)

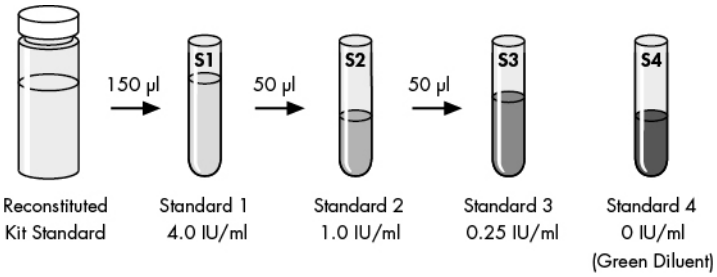

Figure 1. Preparation of standard curve by serial dilution.

4. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml 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 (see Table 1, next page).

   Mix thoroughly but gently, to avoid frothing.

   Return any unused Conjugate 100x Concentrate to 2–8°C immediately after use.

   Use only Green Diluent.

Table 1. Working strength 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 frozen or stored for more than 24 hours prior to assay, thoroughly mix before addition to the ELISA well.

   Important: If plasma samples are to be added directly from the centrifuged QF-CMV Blood Collection Tubes, any mixing of the plasma should be avoided. Always take care not to disturb material on the surface of the gel.

6. If quantitative results are required, dilute CMV and Mitogen plasmas 1/10 in Green Diluent (10 µl plasma + 90 µl GD). The Nil plasma should not be diluted.

   It is recommended that the following samples are tested in parallel:

   Nil, CMV Antigen, Mitogen, CMV Antigen (1/10), Mitogen (1/10)

   However, the following patient sample options are also supported by the QuantiFERON-CMV Analysis Software:

   Nil, CMV Antigen, Mitogen

   Nil, CMV Antigen (1/10), Mitogen (1/10)

   Nil, CMV Antigen, Mitogen, CMV Antigen (1/10)

   Nil, CMV Antigen (1/10), Mitogen

7. Add 50 µl freshly prepared working strength conjugate to the required ELISA wells using a multichannel pipette.
8. Add 50 µl test plasma samples to appropriate wells. Finally, add 50 µl each of the Standards 1 to 4 to the appropriate wells. The standards should be assayed in at least duplicate.
9. Cover the ELISA plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute at 500 to 1000 rpm. Avoid splashing.
10. Cover the ELISA plate and incubate at room temperature (22°C ± 5°C) for 120 ± 5 minutes.

    Plates should not be exposed to direct sunlight during incubation. Deviation from specified temperature range can lead to erroneous results.
11. During the incubation, prepare working strength wash buffer. 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.
12. When ELISA plate incubation is complete, wash wells with 400 µl working strength wash buffer for at least six cycles. An automated plate washer is recommended.

    Important: Thorough washing is very important to the performance of the assay. Ensure 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 followed for the decontamination of potentially infectious material.
13. Tap plates face down on absorbent, lint-free towel to remove residual wash buffer. Add 100 µl Enzyme Substrate Solution to each well, cover the plate and mix thoroughly using a microplate shaker for 1 minute at 500 to 1000 rpm using a microplate shaker.
14. 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.

15. Following the 30-minute incubation, add 50 µl Enzyme Stopping Solution to each well in the same order as the substrate was added and mix thoroughly at 500 to 1000 rpm using a microplate shaker.
16. 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

QuantiFERON-CMV Analysis Software, for the analysis of raw data and calculation of results, is available from QIAGEN at www.QuantiFERON.com. Ensure that the most current version of the QF-CMV 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 "Interpretation of Results" on page 24. The software reports the lowest dilution that generates a result within the range of the QF-CMV ELISA, taking the dilution factor into account.

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

Generation of standard curve (if QF-CMV 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.

The reported result should be taken from the lowest dilution that generates a result within the range of the QF-CMV ELISA, ensure the dilution factor is taken into account where applicable.

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 Standards 3 and 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 QF-CMV 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

QuantiFERON-CMV results are interpreted using the criteria in Table 2.

Table 2. Interpretation of QuantiFERON-CMV results

Nil (IU/ml)	CMV minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QF-CMV result	Report/Interpretation
≤8.0	≥0.20 and ≥25% of Nil	Any	Reactive†	Anti-CMV immunity detected
	<0.20 OR ≥0.20 and <25% of Nil	≥0.5	Nonreactive	Anti-CMV immunity NOT detected
		<0.5	Indeterminate‡	Results are indeterminate for CMV responsiveness
>8.0§	Any	Any	Indeterminate‡	Results are indeterminate for CMV responsiveness

* Responses to the Mitogen positive control (and occasionally CMV antigens) can be commonly outside the range of the microplate reader. This has no impact on test results.

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

‡ Refer to "Troubleshooting Guide" (page 39) for possible causes.

In clinical studies (1), an indeterminate result among solid organ transplant patients, where a donor is reactive for CMV but the Mitogen control was below 0.5 IU/ml, has been shown to be clinically relevant. Such patients have the highest risk of developing CMV.

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

Note: The measured IFN-γ level should be used in conjunction with clinical presentation, medical history and other diagnostic evaluations when establishing the immune response to CMV antigens. QF-CMV is not a test for determining CMV infection and should not be used to exclude CMV infection.

Limitations

Results of QuantiFERON-CMV testing must be used in conjunction with each subject's epidemiological history, current medical status and other diagnostic evaluations.

Unreliable or indeterminate results may occur due to:

- Deviation from the procedure described in the QuantiFERON-CMV ELISA Package Insert
- Excessive levels of IFN-γ in control tube
- Longer than 16 hours between drawing the blood specimen and incubation at 37°C.

Expected Values

Expected IFN-γ values using QuantiFERON-CMV were obtained from testing 591 samples from healthy subjects. 343 samples tested seropositive and 248 samples tested seronegative to CMV IgG. CMV serology status was unknown at the time of QF-CMV testing. In the 248 samples from CMV-seronegative subjects, 100% (248/248) of samples tested were nonreactive by QF-CMV ELISA producing IFN-γ responses of <0.2 IU/ml to the CMV Antigen tube (Nil subtracted). The distribution of IFN-γ responses to CMV Antigen tube (Nil subtracted) for the 343 CMV seropositive subjects is shown (Figure 2).

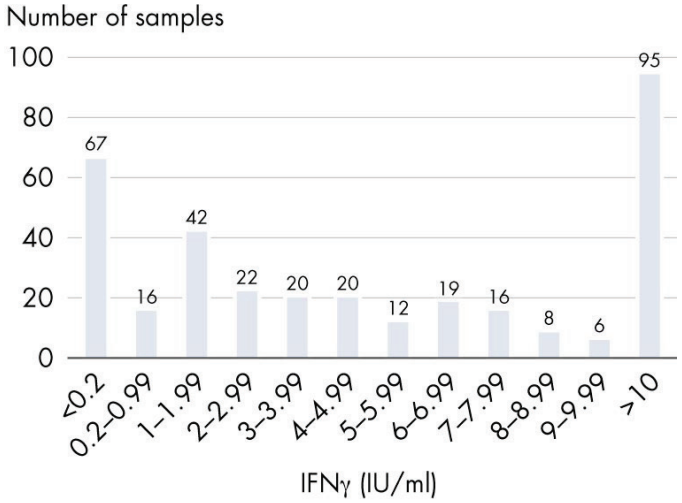

Figure 2. Distribution of QF-CMV IFN-γ responses (Nil subtracted) in seropositive healthy subjects (n = 343).

The distribution of IFN-γ responses to Mitogen (Nil subtracted) was determined using 733 samples from healthy adult subjects using the QF-CMV ELISA, irrespective CMV IgG serology (Figure 3). A Mitogen result (Nil subtracted) of less than 0.5 IU/ml indicates either test failure or that the subject is in an immunocompromised state. In a healthy population, only 2/733 results fell into this category.

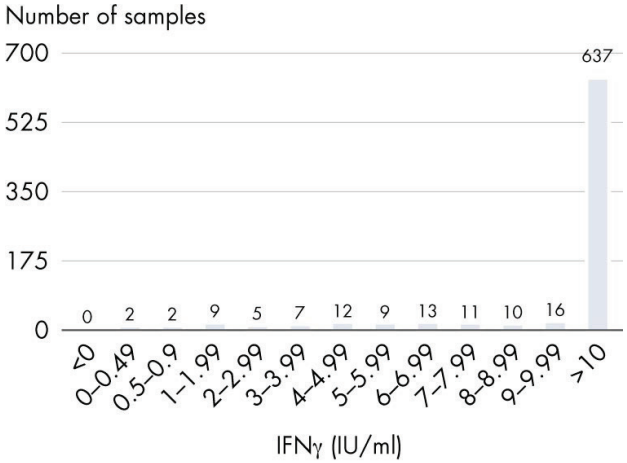

Figure 3. Distribution of Mitogen-IFN-γ responses (Nil subtracted) in healthy subjects (n = 733).

The distribution of IFN-γ responses to Nil tubes were determined using 1020 plasma samples from healthy subjects using the QF-CMV ELISA, irrespective of CMV IgG serology (Figure 4).

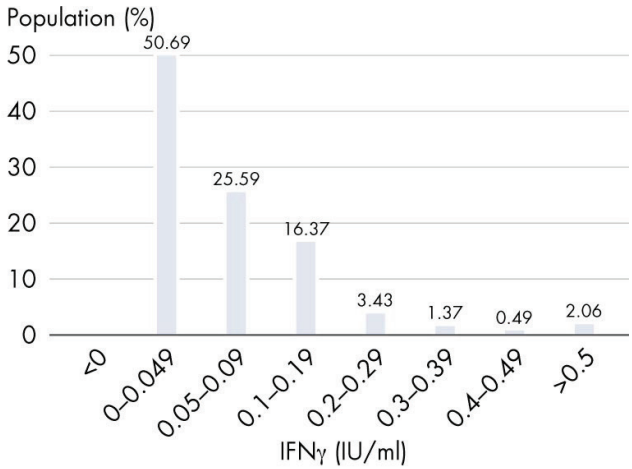

Figure 4. Distribution of Nil IFN-γ responses in healthy subjects (n = 1020) expressed as a percentage of population.

Performance Characteristics

Clinical performance

A test threshold for detecting prior CMV exposure using QF-CMV was established following the analysis of results from a group of healthy subjects (n = 223) in which QF-CMV results were compared to CMV IgG serological results. A ROC analysis determined that a test threshold of 0.04 IU/ml (after Nil subtraction) provided optimal positive and negative predictive values for QF-CMV (area under the curve = 0.9679 [95%CI: 0.9442–0.9915, p<0.0001]), and thus represented the threshold at which this assay performed its intended use most effectively in a healthy population.

The performance of the QF-CMV assay was compared to the SeraQuest™ CMV IgG serology test (Quest International). The QF-CMV assay showed 95% (294/310 individuals) agreement with the CMV IgG serology test in healthy subjects, with none of the 149 seronegative donors showing any reactivity by QF-CMV. 145 of 161 seropositive donors demonstrated a QF-CMV reactive response. Overall positive agreement was 90% with a negative agreement value of 100%. The level of agreement in healthy subjects between QF-CMV responses and the CMV IgG serology status is shown in Table 3.

Table 3. Agreement between QuantiFERON-CMV and CMV IgG serology test in healthy subjects

		CMV serology		
		Positive	Negative	Total
QuantiFERON-CMV	Reactive	145	0	145 (46.8%)
	Non-reactive	16	149	165 (53.2%)
	Total	161 (51.9%)	149 (48.1%)	310 (100%)

Assay threshold

The recommended clinical test threshold for this assay is 0.2 IU/ml in the CMV Antigen tube (Nil subtracted), although different thresholds may be validated for different clinical settings.

Clinical Studies

As there is no definitive standard for confirming or excluding the diagnosis of cytomegalovirus infection, an estimate of sensitivity and specificity for QF-CMV cannot be practically evaluated. The specificity and sensitivity of QF-CMV was approximated by evaluating the level of agreement between QF-CMV responses and the CMV IgG serology status of healthy subjects.

Specificity of QF-CMV was approximated by evaluating false-positive rates (QF-CMV reactive response) in healthy donor samples with no evidence of prior CMV exposure (CMV IgG seronegative individuals). Sensitivity was approximated by evaluating QF-CMV responsiveness in healthy donor samples with evidence of prior CMV exposure (CMV IgG seropositive individuals). QF-CMV utilizes a large number of CMV-specific epitopes from different CMV proteins, and thus provides broad coverage of the population with diverse HLA Class I haplotypes (approximately 98% of the population). As the HLA haplotypes of subjects tested against CMV serology were unknown, a small percentage of serology-positive individuals were expected to be nonresponsive to the QF-CMV Blood Collection Tubes.

Specificity

In a study of 591 samples from healthy subjects, no false-positive QF-CMV results were detected in individuals testing seronegative for CMV IgG with 248/248 samples testing nonreactive by QF-CMV ELISA and negative by CMV IgG serology test. Therefore, the results obtained using QF-CMV and the CMV IgG serology test showed 100% concordance.

In all other specificity evaluations conducted in recipients of solid organ transplants (1–8), recipients of hematopoietic stem cell transplants (9,10) and HIV-infected patients (11), the concordance between QF-CMV and CMV IgG serology has also been shown to be 100%.

Sensitivity

In a study conducted in 343 samples from healthy subjects testing seropositive for CMV IgG, the level of agreement between QF-CMV responses and CMV IgG serology results was 80.5% with 276/343 samples testing reactive to QF-CMV and positive to the CMV IgG serology test. The observed discordance may be due to false-positive CMV serology, or the absence of responsive HLA types in the individuals tested.

The levels of agreement in sensitivity evaluations conducted in solid organ transplant recipients (1–8), hematopoietic stem cell transplant recipients (9, 10) and HIV-infected patients (11), have been shown to be lower and may be due to false-positive CMV serology, the absence of responsive HLA types in the individuals tested, or the absence of reactive T cells in these patients due to their immunosuppression.

Studies highlighting clinical utility

Both CMV IgG serology and QF-CMV describe their intended use as allowing the detection of immunity to CMV. Within a transplant setting, CMV serology is widely used pre-transplantation to establish risk of CMV complications occurring in the recipient post-transplantation, but has limited value itself post-transplantation. Alternatively, QF-CMV may be used in transplant recipients to assess the level of CMV immunity in those patients at risk of developing symptomatic CMV infection and/or disease due to immunosuppression (12–15).

A number of published clinical studies in a variety of transplant cohorts have demonstrated the utility of QuantiFERON-CMV (1–11, 15, 16).

In a large study of 108 solid organ transplant recipients (4), patients with a QF-CMV reactive result at the completion of anti-CMV prophylaxis had a significantly lower rate of subsequent CMV disease (3.3% or 1/30; using an 0.2 IU/ml threshold) compared to patients having a QF-CMV nonreactive result (21.8% or 17/78, p = 0.044) (Figure 5).

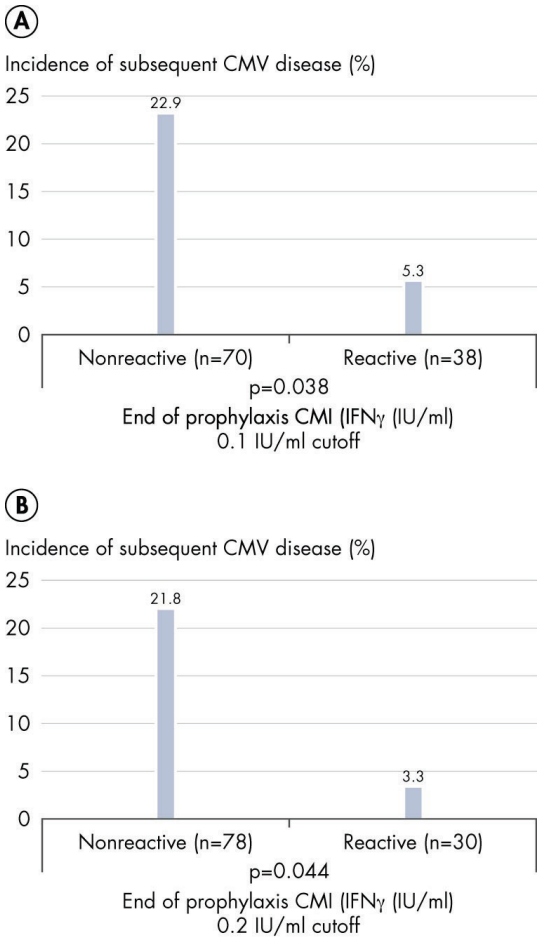

Figure 5. Rates of late-onset CMV disease in patients with a QuantiFERON-CMV reactive result versus a QuantiFERON-CMV nonreactive result at the end of prophylaxis. Underlying data found in Kumar et al. (4).

Furthermore, CMV seronegative transplantation recipients who receive an organ from a CMV-positive donor (D+R–) with a QF-CMV reactive result upon completion of prophylaxis remained free from CMV disease more often, and for longer, indicating that QF-CMV may be used to identify those at risk of developing late-onset CMV disease.

This study also highlighted that in this cohort of transplantation patients at highest risk of developing CMV disease (D+/R–) a reactive result any time post-prophylaxis was associated with a higher chance of remaining free from CMV disease.

In a study of 37 solid organ transplant patients (6), the assessment of CMV specific $CD8^+$ T-cell responses by QF-CMV assisted the prediction of spontaneous viral clearance compared to CMV disease progression, following elevations in CMV viremia. In this study, 24/26 patients (92.3%) with a QF-CMV reactive result (using an IFN-γ $\geq$0.2 IU/ml test threshold), spontaneously cleared CMV virus while only 5/11 (45.5%) patients with a QF-CMV nonreactive result had the same outcome.

A study of 67 lung transplant recipients assessing CMV viremia episodes post-transplant (7), observed that 18/25 (72%) CMV viremia episodes were preceded by a QF-CMV nonreactive result, versus 4/16 (25%) episodes that were preceded by a QF-CMV reactive response (Fisher's exact test, $p = 0.0046$; Figure 6).

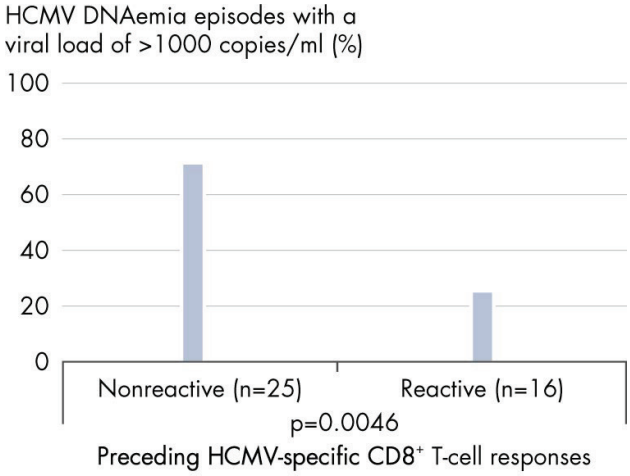

Figure 6. Statistical analysis of CMV-specific CD8+ T- cell responses as detected by QuantiFERON-CMV and the development of CMV viremia (Fisher's exact test, p=0.0046). Underlying data found in Weseslindtner et al (7).

In a large multicenter prospective study of 127 donor CMV-seropositive, recipient CMV-seronegative solid organ transplant patients (8), all of whom received antiviral prophylaxis, showed that patients with a QF-CMV reactive result (using a 0.1 IU/ml test threshold) at any time point following the completion of anti-CMV prophylaxis had a significantly lower rate of late-onset disease at 12 months post-transplant (6.4%), compared with those having a QF-CMV nonreactive result (22.2%) and an indeterminate result (58.3%, $p < 0.001$). When classifying indeterminate results as also being "nonreactive", the incidence of subsequent CMV disease was 6.4% vs. 26.8%, $p = 0.024$. The positive and negative predictive values of QF-CMV for protection from CMV disease were reported to be 0.90 (95% CI 0.74–0.98) and 0.27 (95% CI 0.18–0.37), respectively. This study found that QF-CMV may be useful to predict if patients are at low, intermediate, or high risk for the development of subsequent CMV disease after prophylaxis.

In a prospective study of 55 solid organ transplant recipients (8) where the relationship between pre-transplant QF-CMV results and post-transplant CMV replication episodes were analyzed, it was found that a higher incidence of post-transplant CMV replication was observed in CMV seropositive recipients with a nonreactive (using a 0.2 IU/ml test threshold) pre-transplant QF-CMV result (7/14 or 50%), compared to those CMV seropositive recipients with a reactive pre-transplant QF-CMV result (4/30 or 13.3%, p = 0.021).

This study found that recipients with a nonreactive pre-transplant QF-CMV response who received an organ from a CMV-seropositive donor had a tenfold increased risk of CMV replication compared to those recipients with a reactive pre-transplant QF-CMV response (adjusted OR 10.49, 95% CI 1.88–58.46). Therefore, a pre-transplant QF-CMV assay may be useful in predicting the risk for CMV replication after transplantation and thus allow the individualization of CMV infection management after solid organ transplantation.

A number of other studies investigating the detection of CMV-specific $CD8^+$ T-cell responses by QF-CMV in a cohort of transplant recipients have been completed (2, 3, 5, 9, 10, 15, 16) or are currently in progress worldwide.

International consensus guidelines on the management of cytomegalovirus in solid organ transplant

The importance of CMV-specific immune monitoring has been recognized and published in the *Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation* (12). These international guidelines, developed by a panel of experts on CMV and solid organ transplant, convened by The Infectious Diseases Section of The Transplantation Society, represent evidence and expert opinion-based consensus guidelines on CMV management including: diagnostics, immunology, prevention and treatment.

These guidelines concluded that "Immune monitoring of CMV-specific T-cell responses may predict individuals at risk of CMV disease post-transplant and may be useful in guiding prophylaxis and pre-emptive therapies" (12).

Furthermore, the guidelines also provided recommendations regarding the attributes of the ideal immune monitoring assay, which included:

- Ability to assess the quantity and function of a transplant recipient's $CD4^+$ and $CD8^+$ T cells
- Ability to measure IFN-γ
- Simple to perform, cost-effective and reproducible
- Have a rapid turnaround time
- Allow for specimens to be easily shipped to specialized referral laboratories

QF-CMV meets virtually all the criteria specified by these guidelines and represents the only standardized, immune monitoring assay capable of detecting IFN-γ, specific for CMV.

Assay Performance Characteristics

The QF-CMV ELISA uses recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535). Results for test samples are reported in International Units (IU) relative to a standard curve prepared by testing dilution of the secondary 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 QF-CMV 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 wells.

The limit of detection of the QF-CMV 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. The QF-CMV ELISA antibodies have been shown to not cross-react with any cytokines tested, including IL2, IL3, IL4, IL5, IL6, IL10 and IL12.

The QF-CMV ELISA has been demonstrated to be linear by placing five 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 7).

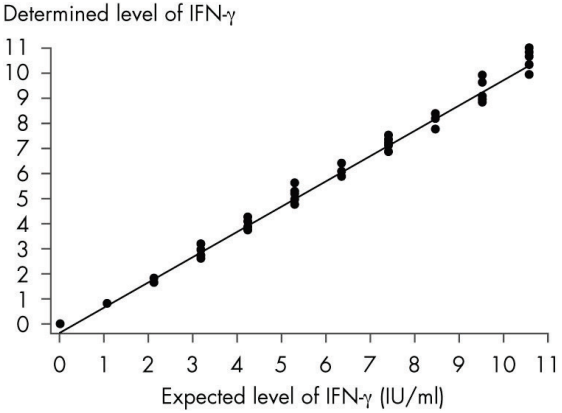

Figure 7. Linearity profile of QF-CMV ELISA determined from testing five replicates of 11 plasma samples of known IFN-γ concentrations.

The reproducibility of the QF-CMV ELISA was estimated by testing 20 plasma samples with varying IFN-γ concentrations in replicates of three, in three laboratories, on three nonconsecutive days, by three operators. Thus, each sample was tested 27 times, in nine independent assay runs. One sample was a Nil control and had a calculated IFN-γ concentration of 0.08 (95% CI 0.07–0.09) IU/ml. Of the remaining 19 plasma samples, the range of concentrations was 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 ranged from 4.1 to 9.1%CV. The average within run %CV (±95% CI) was 6.6% ± 0.6%. The zero IFN-γ plasma averaged 14.1%CV.

Total or inter-assay imprecision was determined by comparing the 27 calculated concentrations of IFN-γ for each plasma sample and 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.

Technical Information

Indeterminate results

Indeterminate results may be related to the immune status of the individual being tested, but may also be related to a number of technical factors:

- Longer than 16 hours from blood draw to incubation at 37°C
- Storage of blood outside the recommended temperature range (22°C ± 5°C)
- Insufficient mixing of blood collection tubes
- Incomplete washing of the ELISA plate

If technical issues are suspected with the collection or handling of blood samples, repeat the entire QF-CMV test with new blood specimens. Repeating the ELISA testing of stimulated plasmas can be performed if any procedural deviation with the ELISA test is suspected. Indeterminate results (from low Mitogen values) would not be expected to change on repeat unless there was an error with the ELISA testing.

Clotted plasma samples

Should fibrin clots occur with long-term storage of plasma samples, centrifuge 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 the back cover.

	Comments and suggestions
Low optical density readings for standards	
a) Standard dilution error	Ensure dilutions of the Kit Standard are prepared correctly as per the QF-CMV ELISA Package Insert.
b) Pipetting error	Ensure pipettes 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.
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 1 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.
Nonspecific color development	
a) Incomplete washing of the plate	Wash the plate at least six times with 400 µl/well of wash buffer. More than six 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 3 months of the reconstitution date.

		Comments and suggestions
d)	Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e)	Mixing of plasma in centrifuge tubes before harvesting	Ensure that plasma samples are carefully harvested from above gel without pipetting up and down, taking care not to disturb material on the surface of the gel.
High background		
a)	Incomplete washing of the plate	Wash the plate at least six times with 400 µl/well of wash buffer. More than six 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		
a)	Incomplete washing of the plate	Wash the plate at least six times with 400 µl/well of wash buffer. More than six 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 kit 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 setup	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
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	CE mark
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number
	Global Trade Item Number
	Temperature limitation
	Do not reuse
	Keep away from sunlight
	Consult instructions for use
	Manufacturer
	Authorized representative in the European Community

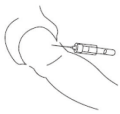
Contact Information

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

Abbreviated ELISA 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, to 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 2000–3000 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

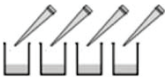
1. Equilibrate ELISA components, with the exception of the Conjugate 100x Concentrate, to room temperature for at least 60 minutes.

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

3. Reconstitute lyophilized Conjugate 100x Concentrate with distilled or deionized water.

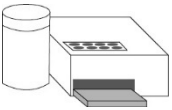
4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.

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

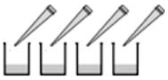


6. Incubate for 120 minutes at room temperature.

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



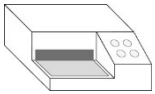
8. Add 100 µl Enzyme Substrate Solution to wells. Mix using shaker.



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.

Handbook Revision History

Document	Changes	Date
L1075110- R06	Materials required but not provided, added plate lids, page 9	October 2019

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