February 2024

QuantiFERON®-TB Gold Plus ELISA Kit Summary of Safety and Performance

2 x 96 (622120)

Version 1

IVD

For In Vitro Diagnostic Use

For use with QuantiFERON®-TB Gold Plus Blood Collection Tubes

CE 0197

REF 622120

QIAGEN GmbH QIAGEN Strasse 1, 40724 Hilden, Germany

R2 MAT

Sample to Insight

Summary of Safety and Performance

This Summary of Safety and Performance (SSP) is intended to provide public access to an up-to-date summary of the main aspects of the safety and performance of the device.

The SSP is not intended to replace the Instructions For Use as the main document to ensure the safe use of the device, nor is it intended to provide diagnostic or therapeutic suggestions to intended users.

The following information is intended for professional users.

Document revision: Rev.02
Date issued: February 2024 Rev.02
Manufacturer's reference number for the SSP: n/a

1. Device identification and general information	
1.1 Device trade name(s)	Fourth generation of the QuantiFERON-TB technology QuantiFERON®-TB Gold Plus (QFT-Plus) 622120 QuantiFERON-TB Gold Plus 2 Plate Kit ELISA 622822 QuantiFERON-TB Gold Plus Reference Lab Pack QuantiFERON-TB Gold Plus (QFT-Plus) Blood Collection Tubes 622423 QFT-Plus Dispenser Pack (25ct) 622526 QFT-Plus Tubes (50x TB1/TB2/Nil/Mitogen) 622222 QFT-Plus) Single Patient Pack (Pack of 10) 623423 QFT-Plus HA Dispenser Pack (25ct) 623526 QFT-Plus HA tubes (50x TB1/TB2/Nil/Mitogen) 623222 QFT-Plus HA Single Patient Pack (pack of 10)
1.2 Manufacturer's name and address	QIAGEN GmbH QIAGEN Strasse 1, 40724 Hilden, Germany
1.3 Manufacturer's single registration number (SRN)	DE-MF-000004949
1. 4 Basic UDI-DI	4053228RTBQFT0000000001W8 (QFT ELISA) 4053228RTBQFT0000000002WA (QFT Tubes)
1.5 European Medical Device Nomenclature (EMDN) description / text	EMDN code (5th level): W01050107, MYCOBACTERIA GENUS + SPECIES (QFT ELISA) EMDN code (5th level): W05010101, VENOUS OR ARTERIOUS BLOOD COLLECTION DEVICES (QFT Tubes)
1.6 Risk Class of the device	Class C
1.7 Indication whether it is a device for near-patient testing and/or a	QuantiFERON®-TB Gold Plus is not a device for near-patient testing nor companion diagnostic test.

companion diagnostic	
1.8 Year when the first certificate was issued under Regulation (EU) 2017/746 covering the device	QuantiFERON-TB Gold Plus has been certified under the EU Regulation 2017/746 in 2023.
1.9 Authorised representative if applicable; name and the SRN	Not applicable
1.10 Notified body and the single identification number (SIN)	TÜV Rheinland LGA Products GmbH Tillystraße 2 90431 Nürnberg Germany TÜV: 0197
2. Intended use of the device	
2.1 Intended purpose	The QuantiFERON-TB Gold Plus (QFT-Plus) assay is an *in vitro* diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of Interferon gamma (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify in vitro responses to those 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.
2.2 Indication(s) and target population(s)	LTBI testing is desirable whenever feasible to identify persons at high-risk for developing active TB so TB preventive treatment could be considered. Based on WHO recommendations: (**https://apps.who.int/iris/bitstream/handle/10665/331170/9789240001503-eng.pdf**), LTBI testing is required for high risk groups including but not limited to household contacts over than 5 years old,

	patients with silicosis, those on haemodialysis, anti-TNF agent treatment, preparation for transplantation as well as other risks groups according to national guidelines.
2.3 Limitations and/or contra-indications	• Results from QFT-Plus testing must be used in conjunction with each individual's epidemiological history, current medical status, and other diagnostic evaluations. • Individuals with Nil values greater than 8 IU/ml are classed as "Indeterminate" because a 25% higher response to TB Antigens may be outside the assay measurement range. • The predictive value of a positive QFT-Plus result in diagnosing M. tuberculosis infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic, and other findings. • A diagnosis of LTBI requires that tuberculosis disease must be excluded by medical evaluation including an assessment of current medical and diagnostic tests for disease as indicated. • A negative result must be considered with the individual's medical and historical data relevant to probability of M. tuberculosis infection and potential risk of progression to tuberculosis disease, particularly for individuals with impaired immune function. • Unreliable or indeterminate results may occur due to deviations from the procedure described in the package insert o Incorrect transport/handling of blood specimens. o Elevated levels of circulating IFN-γ or presence of heterophile antibodies. o Exceeding validated blood times from blood specimen draw to incubation.
3. Device description	
3.1 Description of the device, including the conditions to use the device	The QuantiFERON®-TB Gold Plus (QFT-Plus) assay is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 and CFP-10 proteins to stimulate cells in heparinized whole blood. Detection of Interferon-γ (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify in vitro responses to those 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. This kit is intended for professional use. The QuantiFERON®-TB Gold Plus (QFT-Plus) assay is to be used by trained personnel in a professional laboratory environment or by a trained phlebotomist. QuantiFERON®-TB Gold Plus (QFT-Plus) test is the fourth generation in QuantiFERON-TB testing technology assessing cell-mediated response through a quantitative measurement of IFN-γ in a whole blood sample. QFT-Plus is a qualitative test that measures the cell-mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii, M. szulgai, and M. marinum*. Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial 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. QuantiFERON®-TB Gold Plus (QFT-Plus) Blood Collection Tubes are intended for the collection, storage, incubation, stimulation, and transportation of human blood. The QFT-Plus is a qualitative assay that uses specialized blood collection tubes, containing peptide antigens that simulate M. tuberculosis proteins, which are used to collect whole blood. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens. Whole blood is collected into each of the QFT-Plus Blood Collection Tubes, which include a Nil tube, TB1 tube, TB2 tube, and Mitogen tube. Alternatively, blood may be collected in a single blood collection tube that contains lithium- or sodium-heparin as the anticoagulant and then transferred to QFT-Plus Blood Collection Tubes. The software is optional for use with the device.

	The software performs a Quality Control assessment of the assay, generates a standard curve, and provides a test result for each subject. The software reports all concentrations greater than 10 IU/ml as ">10" as such values fall beyond the validated linear range of the ELISA.
3.2 In case the device is a kit, description of the components (including regulatory status of components, for example, IVDs, medical devices and any Basic UDI-DIs)	The QFT-Plus ELISA is sold in both a 2-plate kit with components and a reference lab pack that contains 20 plate and components. The QFT-Plus BCTs are sold in packs of 200 tubes (50 Nil, 50 TB1, 50 TB2, and 50 Mitogen tubes), 100 tubes (25 of each type of tube), or in single patient packs (10 individual packs that each contain 1 Nil, 1 TB1, 1 TB2 and 1 Mitogen tubes). QFT-Plus high altitude BCTs are also available in the configurations shown as above. Description of the components of the device. • Microplate strips (12 x 8 wells) • IFN-γ Standard, lyophilized • Green Diluent • Conjugate 100x Concentrate, lyophilized • Wash Buffer, 20x Concentrate • Enzyme Substrate Solution • Enzyme Stopping Solution
3.3 A reference to previous generation(s) or variants if such exists, and a description of the differences	QuantiFERON® TB Gold In Tube (QFT) is the 3rd generation assay, it is a three tube assay that has peptides designed to stimulate only MTB-specific CD4 T cells. 1. Nil- Negative Control 2. TB Antigen- Primarily detects MTB-specific CD4 T cell responses 3. Mitogen- Positive Control QFT Plus assay uses a proprietary combination of peptides designed for contraindications and activity. QFT Plus is a four tube assay that has two TB tubes for detection of MTB specific cell-mediated response: 1. Nil- Negative Control 2. TB1- Primarily detects MTB-specific CD4 T cell response 3. TB2- Optimized for detection of MTB-specific CD4 and CD8 T cell responses 4. Mitogen- Positive Control

3.4 Description of accessories intended to be used in combination with the device	Not applicable - QFT-Plus is a standalone assay.
3.5 Description of any other devices and products which are intended to be used in combination with the device	Not applicable - QFT-Plus is a standalone assay.
4. Reference to any harmonised standards and CS applied	
4 Harmonised standards and Common Specifications (CS) applied	The relevant harmonized standards have been followed to support the performance evaluation as applicable for QFT-Plus. Harmonized standards (EN): • EN ISO 13612:2002+AC:2002 Performance evaluation of in vitro diagnostic medical devices • EN ISO 14971:2019, EN ISO 14971:2019/A11:2021 Medical Devices – Application of risk management to medical devices • ISO 13485 2016/AC:2018/A11:2021 (Medical devices - Quality management systems - -Requirements for regulatory purposes) • EN ISO 17511:2021 In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials • EN ISO 18153:2003 In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values for catalytic concentration of enzymes assigned calibrators and control materials • EN ISO 23640:2015 In vitro diagnostic medical devices. Evaluation of stability of in vitro diagnostic reagents • EN ISO/DIS 20916 IVD medical devices - Clinical performance studies using specimens from human subjects – Good study practice

	Standards (CLSI): • CLSI EP5-A3 Evaluation of Precision Performance of Quantitative Measurement Methods • CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures • CLSI EP07-A2 Interference Testing in Clinical Chemistry • CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance • CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures • CLSI EP24-A2 Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Curves • CLSI EP-25-A Evaluation of Stability of In Vitro Diagnostic Reagents
5. Risks and warnings	
5.1 Residual risks and undesirable effects	Risks have been mitigated as far as possible and deemed as acceptable, The Instruction for Use ("Warnings and Precautions" and "Limitation") provides warnings for the residual risks and any precautions to keep these risks under control. The current residual risks are acceptable. The information and instructions provided by the manufacturer are easy for the intended user to understand and apply, to correctly interpret the result provided by the device and to avoid misleading information. Results from the QFT-Plus testing must be used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluation. Individuals with Nil values greater than 8 IU/ml are classed as "Indeterminate" because a 25% higher response to CMV Antigens may be outside the assay measurement range. • A negative QFT-Plus result does not preclude the possibility of M. tuberculosis infection or TB disease: false negative results can be due to stage of infection (e.g., specimen obtained prior to the development of cellular response), co-morbid conditions that affect immune function, incorrect handling of

	the blood collection tubes following venipuncture, incorrect performance of the assay, or other immunological variables. Unreliable or indeterminate results may occur due to: • Deviations from the procedure described in the package insert • Incorrect transport/handling of blood specimen • Elevated levels of circulating IFN-γ or presence of heterophile antibodies • Exceeding validated blood times from blood specimen draw to incubation.
5.2 Warnings and precautions	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. If you suspect the QFT-Plus Blood Collection Tube(s) have been damaged or sterilization has been compromised, contact QIAGEN Technical Services. Thimerosal is used as a preservative in some QFT-Plus reagents. It may be toxic upon ingestion, inhalation or skin contact. 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) available online in convenient and compact PDF format to view and print at **www.qiagen.com/safety**. 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 Substate Solution: Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection. QuantiFERON Green Diluent: Contains: tartrazine. Warning! May cause an allergic skin reaction. Wear protective gloves/ protective clothing/ eye protection/ face protection. • The reconstituted kit standard may be kept for up to 3 months if stored at 2°C to 8°C. Note the date the kit standard was reconstituted. • The reconstituted Conjugate 100X Concentrate must be returned to storage at 2 to 8°C and must also be used within 3 months. Note the date 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 for up to 2 weeks.
5.3 Other relevant aspects of safety, including a summary of any field safety corrective action (FSCA including FSN), if applicable	There have been no Field Safety Corrective Actions for QFT TB Plus. No new hazards have been identified for this product.

6. Summary of the performance evaluation and post-market performance follow-up (PMPF)	
6.1 Summary of scientific validity of the device	The QFT-Plus assay, including previous generations, measures the production of IFN-γ by MTB-specific T cells to identify in vitro responses to the antigens that are associated with MTB infection. Below is a summary of the scientific foundation for QFT-Plus, connecting the production of the analyte IFN-γ by T cells upon exposure to MTB antigens to the detection of the clinical condition, MTB infection (TBI). Current national and international recommendations recognize the critical importance of TBI screening as a key factor for TB incidence reduction and elimination. Since TBI is a non-infectious state, it can only be detected using indirect immunological methods. Two main methodologies for LTBI diagnosis include Tuberculin skin tests (TST) and Interferon-gamma releasing assays (IGRA) [WHO Global Tuberculosis Report 2023 **https://www.who.int/publications/i/item/9789240083851**. QFT-Plus is the most recognized IGRA for TBI diagnosis in the world. Many publications demonstrate its excellent performance in high-risk groups and as of October 2023, over 100 million tests were used worldwide. Specifically, excellent performance (high sensitivity and specificity) of QFT-Plus was demonstrated for principal high risk groups including children, people living with HIV, those on immunosuppressive therapy, migrants, active TB contacts etc [1, 2, 3, 4]. Excellent performance of QFT-Plus in various high risk groups including children has been confirmed in original studies as well as systematic and narrative reviews [5]. QFT-Plus has been recommended by both the World Health Organization (WHO 2020, WHO, M3 2021, WHO, M5, 2022) [6,7,8] and Centers for Disease Control and Prevention (CDC) as well as European centre for Disease Control (ECDC) [9]. International bodies' recommendations were based on multiple publications including original papers and systematic reviews demonstrating excellent performance of QFT-Plus in various populations including WHO-defined risk groups for TB infection and TB reactivation.

	Published studies demonstrate that the QFT-Plus assay has higher sensitivity in household contacts and in immunocompromised persons (HIV, rheumatoid arthritis, elderly, and those with low CD4 T cell counts), demonstrating non-inferior specificity to QFT-GIT (previous generation) [10, 11]. 1. Barcellini L, et al. First independent evaluation of QuantiFERON-TB Plus performance. Eur Respir J. 2016 May;47(5):1587-90. doi: 10.1183/13993003.02033-2015. Epub 2016 Feb 11. PMID: 26869677 2. Fukushima K, Kubo T, Akagi K, et al. Clinical evaluation of QuantiFERON®-TB Gold Plus directly compared with QuantiFERON®-TB Gold In-Tube and T-Spot®.TB for active pulmonary tuberculosis in the elderly. J Infect Chemother. 2021;27(12):1716-1722. doi:10.1016/j.jiac.2021.08.016 3. Ho CS, Feng PI, Narita M, et al. Comparison of three tests for latent tuberculosis infection in high-risk people in the USA: an observational cohort study. Lancet Infect Dis. 2022;22(1):85-96. doi:10.1016/S1473-3099(21)00145-6 4. Igari H, Akutsu N, Ishikawa S, et al. Positivity rate of interferon-γ release assays for estimating the prevalence of latent tuberculosis infection in renal transplant recipients in Japan. J Infect Chemother. 2019;25(7):537-542. doi:10.1016/j.jiac.2019.02.018 5. Ahmed A, Feng PI, Gaensbauer JT, et al. Interferon-γ Release Assays in Children <15 Years of Age [published correction appears in Pediatrics. 2020 May;145(5):]. Pediatrics. 2020;145(1):e20191930. doi:10.1542/peds.2019-1930 6. WHO, M1.2020. 'WHO consolidated guidelines on tuberculosis. Module 1: Prevention'. 7. WHO, M3. 2021. 'WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection 2021 update'. 8. WHO, M5. 2022. 'WHO consolidated guidelines on tuberculosis Module 5: Management of tuberculosis in children and adolescents'.

	9. ECDC. 'Review of reviews and guidelines on target groups, diagnosis, treatment and programmatic issues for implementation of latent tuberculosis management' (September 2018) 10. Siegel SAR, Cavanaugh M, Ku JH, Kawamura LM, Winthrop KL. Specificity of QuantiFERON-TB Plus, a New-Generation Interferon Gamma Release Assay. J Clin Microbiol. 2018 Nov 27;56(12):e00629-18. doi: 10.1128/JCM.00629-18. PMID: 30232132; PMCID: PMC6258840. 11. Sotgiu, G., L. Saderi, E. Petruccioli, S. Aliberti, A. Piana, L. Petrone, and D. Goletti. 2019. 'QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis', *J Infect*, 79: 444-53.
6.2 Summary of performance data from the equivalent device, if applicable	Not Applicable
6.3 Summary of performance data from conducted studies of the device prior to CE-marking	A summary of the analytical and clinical performance studies is provided below: Assay cut-off The QFT-Plus assay cut-off was determined using data from 216 subjects with no identified risk factors for TB exposure, who had been BCG vaccinated and assumed to be free of infection and 118 subjects with culture confirmed M. tuberculosis infection. The sensitivity and specificity data was combined and analyzed by Receiver Operator Characteristic (ROC) curve analysis. The sensitivity and specificity data analyzed using the ROC analysis demonstrated that the optimal ELISA cut-off was 0.35 IU/mL (see Figure 1 Table 1).

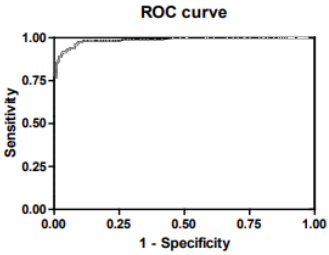

Figure 1. ROC Curve for the ESAT-6 and CFP-10 Responses

Table 1 . Sensitivity and Specificity Values for the ELISA at Various Cut-Offs

Cutoff IU/ml IFN-γ	Sensitivity %	95% CI	Specificity %	95% CI	Sensitivity + Specificity
0.20	91.53	84.97% to 95.86%	96.31	92.87% to 98.40%	187.84
0.23	91.53	84.97% to 95.86%	96.77	93.47% to 98.69%	188.30
0.26	90.68	83.93% to 95.25%	96.77	93.47% to 98.69%	187.45
0.28	90.68	83.93% to 95.25%	97.24	94.08% to 98.98%	187.92
0.30	89.83	82.91% to 94.63%	97.24	94.08% to 98.98%	187.07
0.31	88.98	81.90% to 94.00%	97.24	94.08% to 98.98%	186.22
0.33	88.98	81.90% to 94.00%	97.70	94.71% to 99.25%	186.68
0.35	**88.98**	**81.90% to 94.00%**	**98.16**	**95.35% to 99.50%**	**187.14**
0.39	88.14	80.90% to 93.36%	98.16	95.35% to 99.50%	186.3
0.42	87.29	79.90% to 92.71%	98.16	95.35% to 99.50%	185.45
0.43	86.44	78.92% to 92.05%	98.16	95.35% to 99.50%	184.6
0.45	86.44	78.92% to 92.05%	98.62	96.01% to 99.71%	185.06
0.47	85.59	77.94% to 91.38%	99.08	96.71% to 99.89%	184.67
0.48	84.75	76.97% to 90.70%	99.08	96.71% to 99.89%	183.83
0.50	83.90	76.00% to 90.02%	99.08	96.71% to 99.89%	182.98

Linearity

The QFT-Plus ELISA has been demonstrated to be linear by placing 5 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 2).

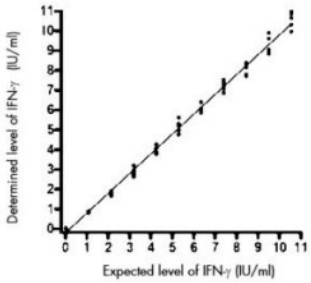

Figure 2. Illustration of Linearity Study Regression Analysis – High Pool Mean = –0.24+0.9964•Expected.

Reproducibility

A multi-center study reproducibility study was conducted to evaluate performance of QFT-Plus across study sites with multiple operators. This was a prospective study conducted at three external testing sites and one collection site. A total of 32 positive and 34 negative (determined by the QFT test) study subjects were enrolled. The study subjects were comprised of healthcare workers in the United States. The study subjects represented groups with mixed risk for TB exposure due to their occupation or as foreign born healthcare workers originating from a location with a TB rate exceeding 50/100,000. Three lithium-heparin blood collection tubes were obtained from each study subject at the collection site. The lithium-heparin blood collection tubes were then transferred to each of three testing sites where they were aliquoted into two sets of QFT-Plus Blood Collection Tubes (QFT-Plus TB1, TB2, Mitogen, and Nil) then tested in accordance with the QFT-Plus assay procedure. At each site at least two operators ran the

two tests per study subject independently. Each operator was blinded to the results obtained by the other operator and blinded to the QFT test result of the study subject. There were six results generated across all three testing sites per each of the 66 study subjects, resulting in a total of 396 data points. A summary of the reproducibility summary results are provided in Table 2.

Table 2. Reproducibility study results summary – within site % agreement of qualitative results between operators; N = 66 patient samples

Site 1 – 2 Operators	Site 2 – 2 Operators	Site 3 – 3 Operators
64/66 = 96.97%	64/66 = 96.97%	59/66 = 89.39%
Agreement of Qualitative Results of Tube Set 1 and Tube Set 2	Agreement of Qualitative Results of Tube Set 1 and Tube Set 2	Agreement of Qualitative Results of Tube Set 1 and Tube Set 2

The qualitative percent agreement across all study sites is 94.7% (375/396). In this calculation, the total number of test results in agreement (375) includes those instances where 54 QuantiFERON®-TB Gold Plus ELISA Instructions for Use there is agreement of all 6 results, agreement of 5 out of 6 results, agreement of 4 out of 6 results, and agreement of 3 out 6 results, combined.

Inter-lot repeatability

A study was conducted to determine the inter-lot variability of QFT-Plus Blood Collection Tubes when compared to the QFT tubes. A total of 30 subjects (15 confirmed TB positive and 15 confirmed TB negative determined by the QFT test) were tested. Three separate lots each of the QFT-Plus TB1, TB2, and QFT TB Blood Collection Tubes were included in this study. Three replicates per donor per blood collection tube lot were tested. Nil and Mitogen tubes were tested with one replicate each. Blood from each subject was collected into lithium-heparin blood collection tubes and then 1 ml of blood was transferred to each of the QFT-Plus and QFT Blood Collection Tubes and tested in accordance with the assay procedure. For each positive

and negative sample group, the total variance of the QFT-Plus tube results must not have been significantly greater than the total variance of the QFT tube results. This was determined from the p-value given by the Levene's Homogeneity of Variance (HOV) test. If the p-value was not significant ($p>0.05$) and/or the variation of the QFT-Plus TB tubes was lower than that of the QFT TB tube, then there was variance between the QFT-Plus and QFT TB tubes.

Table 3. Comparison of Variances between QFT-Plus and QFT TB Blood Collection Tubes using Levene's HOV Test

Sample type	Difference	Effect	Dependent	P-value	Significant
Positive	TB2 vs QFT	Sub_Type	Residual	0.0378	Yes
Positive	TB2 vs QFT	Sub_Type	Residual	0.0540	No
Negative	TB2 vs QFT	Sub_Type	Residual	0.1025	No
Negative	TB2 vs QFT	Sub_Type	Residual	0.6344	No

The variation between the QFT-Plus and QFT TB Blood Collection Tubes was not significant with the exception of the QFT-Plus TB2 tube when tested with positive subjects. When the estimate of standard deviation was analyzed, the variation seen in the QFT-Plus TB2 tube was smaller (0.06089) than the QFT TB tube (0.07641) as shown in Table 4. Therefore, the variance of the QFT-Plus TB1 and TB2 Blood Collection Tubes was no greater than the QFT TB Blood Collection Tube.

Table 4. Standard Deviation for Residual and 95% Confidence Interval for Positive Subjects

Sample type	Sub type	Standard Deviation Estimate	95% LCL	95% UCL
Positive	QFT	0.07641	0.06826	0.08680
Positive	TB1	0.06275	0.05605	0.07127
Positive	TB2	0.06089	0.05439	0.06917

Intra-lot repeatability

A study was conducted to assess the intra-lot reproducibility of the QFT-Plus Blood Collection Tubes by comparing the IFN-γ

concentration from replicates of QFT-Plus TB Blood Collection Tubes of blood. Six aliquots of one blood sample from the same subjects with a confirmed TB infection were run in 6 repeat blood collection tubes from one lot each of both QFT-Plus tubes (TB1 and TB2). The testing was performed in 13 subjects. The %CV was calculated for each donor and across all donors to generate a mean %CV as shown in Table 5.

Table 5. The %CV for Mean, Standard Deviation, Minimum, Median and Maximum in each QFT-Plus TB Blood Collection Tube in TB Positive Subjects.

QFT-Plus Tube	Sample size	Mean(%CV)	Standard Deviation	Minimum	Median	Maximum
TB1	13	13.31	6.88	4.17	12.87	29.56
TB2	13	13.04	7.48	4.86	10.75	29.44

The results demonstrated that the mean %CV for TB1 and TB2 was ~13%, meeting the < 30% acceptance criteria and demonstrating intra-lot repeatability.

Limit of Blank (LoB)

The Limit of Blank (LoB) was evaluated for the QFT-Plus assay. Two replicates each of 14 individual normal human plasma samples (as the blanks) were tested with 2 lots of the QFTPlus ELISA by 3 operators on 3 testing days, one operator per testing day for a total of 84 replicates from each ELISA kit lot. The LoB values (IU/mL) for the 2 ELISA kit lots were calculated separately as shown in Table 6.

Table 6. LoB Values (IU/mL) for the 2 QFT-Plus ELISA Kit Lots

QFT-Plus ELISA Kit	LoB estimated (IU/ml)
Kit 1	0.030
Kit 2	0.040

The larger LoB value, 0.040 IU/mL, across both QFT-Plus ELISA kit lots, was reported as the final LoB value.

Limit of Detection (LoD)

The Limit of Detection (LoD) was evaluated for the QFT-Plus assay. A TB negative human plasma pool was generated by combining 14 individual plasma samples. Each of the 3 operators prepared an IFN-γ reference standard stock at 1.0 IU/mL diluted in buffer. A dilution series of 8 concentrations were made. The study was conducted over 3 days, by 3 alternating operators using 2 QFT-Plus ELISA kit lots. For each testing day, 5 replicates of each concentration within each set of the serial dilution series were tested for a total of 45 replicates for each dilution of IFN-γ concentration for each QFT-Plus ELISA kit lot. The LoD value for each of the QFT-Plus ELISA kit lots tested was calculated separately as shown in Table 7.

Table 7. Estimated LoD Values (IU/mL) for the 2 QFT-Plus ELISA Kit Lots

QFT-Plus ELISA Kit	Probability	Concentration estimate (IU/ml)	Lower 95% Confidence Limit for Estimate	Upper 95% Confidence Limit for Estimate
Kit 1	0.95	0.063	0.060	0.067
Kit2	0.95	0.065	0.060	0.073

Interfering substances

A study was conducted to determine the effects of potential interfering substances on the performance of the QFT-Plus ELISA detection of IFN-γ. The interferents included in this testing were: triglycerides (Total), hemoglobin, protein (total serum), bilirubin (conjugated), bilirubin (unconjugated), Abacavir sulfate, Cyclosporine and Prednisolone. Five plasma pools with known concentrations of IFN-γ were prepared using different interferent concentrations. The base pool IFN-γ level was previously prepared with a pre-determined amount of IFN-γ present (approximately 0.21, 0.45 and 1.4 IU/mL). This pool was then used to prepare the interferent pools. The interferent concentrations tested were 0 mg/dL, 5 mg/dL, 10 mg/dL, 15 mg/dL and 20 mg/dL. The target interferent concentrations were based on

reference intervals, pathological values, therapeutic ranges, and toxic ranges or as recommended by vendor or general clinical levels. Six replicates were tested for each interferent sample concentration level. For each sample concentration, a two-sample t-test was performed, comparing the difference in mean log10 (IU/mL) of the primary interferent level compared to the control (i.e. interferent free level) as shown in Table 8 and 9. The estimated difference in mean response, along with the corresponding two-sided 95% confidence limits and p-value were also reported.

Table 8. Log10 IU/mL: T-Test Summary Table for Differences in Means between Control and Primary Interferent Level for each Interferent and IFN-γ Concentration Level.

Interferent	Interferent level	Sample concentration (IU/ml)	Variances	Mean Difference	Lower 95% CI	Upper 95% CI	P-value	Pass
Triglycerides	High	1.4	Equal	0.019	–0.040	0.077	0.491	Yes
		0.45	Equal	0.004	–0.022	0.030	0.732	Yes
		0.21	Equal	0.006	–0.035	0.047	0.759	Yes
Hemoglobin	High	1.4	Equal	–0.005	–0.42	0.032	0.784	Yes
		0.45	Equal	–0.000	–0.023	0.023	0.981	Yes
		0.21	Equal	0.000	–0.034	0.035	0.980	Yes
Protein	High	1.4	Equal	0.004	–0.034	0.042	0.836	Yes
		0.45	Equal	0.001	–0.38	0.040	0.962	Yes
		0.21	Equal	–0.008	–0.076	0.060	0.809	Yes
Bilirubin Conjugated	High	1.4	Equal	–0.011	–0.057	0.034	0.589	Yes
		0.45	Equal	–0.002	–0.058	0.053	0.923	Yes
		0.21	Equal	–0.014	0.074	0.046	0.625	Yes
Bilirubin Unconjugated	High	1.4	Equal	–0.008	–0.041	0.026	0.614	Yes
		0.45	Equal	–0.000	–0.042	0.041	0.982	Yes
		0.21	Equal	–0.000	–0.048	0.048	0.989	Yes
Abacavir	High	1.4	Equal	0.008	–0.025	0.041	0.601	Yes
		0.45	Equal	0.012	–0.019	0.044	0.412	Yes
		0.21	Equal	–0.006	–0.052	0.040	0.770	Yes

Interferent	Interferent level	Sample concentration (IU/ml)	Variances	Mean difference	Lower 95% CI	Upper 95% CI	P-value	Pass
Cyclosporine	High	1.4	Equal	0.014	–0.020	0.047	0.383	Yes
		0.45	Equal	0.005	–0.035	0.045	0.773	Yes
		0.21	Equal	0.024	–0.008	0.056	0.131	Yes
Prednisolone	High	1.4	Equal	0.017	–0.017	0.050	0.293	Yes
		0.45	Equal	0.000	–0.036	0.036	0.979	Yes
		0.21	Equal	0.015	–0.035	0.065	0.524	Yes

Table 9. Log10 IU/mL: T-Test Summary Table for Differences in Means between Control and High Interferent Level for each Interferent and IFN-γ Concentration Level

Interferent	Interferent level	Sample concentration (IU/ml)	Variances	Mean Difference	Lower 95% CI	Upper 95% CI	P-value	Pass
Triglycerides	High	1.4	Equal	0.053	–0.004	0.110	0.063	Yes
		0.45	Equal	0.039	–0.021	0.058	<.001	Yes
		0.21	Equal	0.034	–0.002	0.071	0.061	Yes
Hemoglobin	High	1.4	Equal	–0.001	–0.042	0.040	0.967	Yes
		0.45	Equal	0.016	–0.007	0.040	0.152	Yes
		0.21	Equal	0.014	–0.030	0.059	0.489	Yes
Protein	High	1.4	Equal	–0.030	–0.071	0.011	0.136	Yes
		0.45	Equal	0.000	–0.046	0.046	0.992	Yes
		0.21	Equal	–0.045	–0.103	0.012	0.109	Yes
Bilirubin Conjugated	High	1.4	Equal	0.001	–0.046	0.048	0.961	Yes
		0.45	Equal	0.012	–0.043	0.067	0.639	Yes
		0.21	Equal	0.015	–0.044	0.074	0.586	Yes
Bilirubin Unconjugated	High	1.4	Equal	0.015	–0.011	0.042	0.231	Yes
		0.45	Equal	0.015	–0.023	0.052	0.411	Yes
		0.21	Equal	0.012	–0.033	0.057	0.566	Yes
Abacavir	High	1.4	Equal	0.013	–0.015	0.040	0.322	Yes
		0.45	Equal	0.015	–0.014	0.044	0.283	Yes
		0.21	Equal	0.008	–0.034	0.050	0.677	Yes

Interferent	Interferent level	Sample concentration (IU/ml)	Variances	Mean difference	Lower 95% CI	Upper 95% CI	P-value	Pass
Cyclosporine	High	1.4	Equal	0.002	–0.019	0.024	0.816	Yes
		0.45	Equal	0.007	–0.030	0.043	0.682	Yes
		0.21	Equal	0.015	–0.007	0.038	0.155	Yes
Prednisolone	High	1.4	Equal	0.007	–0.016	0.030	0.518	Yes
		0.45	Equal	–0.001	–0.034	0.033	0.964	Yes
		0.21	Equal	0.021	–0.025	0.068	0.334	Yes

	The results showed no significant differences between the primary interference level and control (interferent-free level) and for the high interferent level except for the Triglyceride 0.45 IU/mL concentration level. The mean difference was determined to be within the +/- 2 standard deviation range. This demonstrates that the difference is within the expected variability of the assay and that Triglyceride did not have an interfering effect on the QFT-Plus ELISA. Clinical performance Clinical Specificity A multi-center study evaluating the clinical specificity of QFT-Plus was performed in 733 study subjects who were considered to have either low risk of M. tuberculosis infection or no risk factors for exposure to infection or disease. Risk factors for TB exposure were determined using a standardized survey at the time of testing. The study was conducted at 4 independent sites, including 1 in the US, 2 in Japan, and 1 in Australia. QFT-Plus was compared to QuantiFERON®-TB Gold In-Tube (QFT). A summary of the clinical specificity performance data stratified by study site and region is provided in Figure 3. The performance results are based on the total number of valid tests. There were no indeterminate results.

Site	N	Positive		Negative		Indeterminate		Specificity (95% CI)	
		QFT	QFT-Plus	QFT	QFT-Plus	QFT	QFT-Plus	QFT	QFT-Plus
United States									
(#1) USA-4	212	2	4	210	208	0	0	99.06% (210/212) (96.63–99.74)	98.11% (208/212) (95.25–99.26)
Japan									
(#2) JPN-3	106	1	2	105	104	0	0	99.06% (105/106) (94.85–99.83)	98.11% (104/106) (93.38–99.48)
(#3) JPN-1	216	3	5	213	211	0	0	98.61% (213/216) (96.00–99.53)	97.69% (211/216) (94.70–99.01)
Total Japan	322	4	7	318	315	0	0	98.76% (318/322) (96.85–99.52)	97.83% (315/322) (95.6–98.9)
Australia									
(#4) AU-3	199	8	9	191	190	0	0	95.98% (191/199) (92.27–97.95)	95.48% (190/199) (91.63–97.60)

Figure 3. Specificity of QFT-Plus

The specificity of QFT-Plus was 98.11% in the US, 97.83% in Japan, and 95.48% in Australia. The overall specificity of QFT-Plus was 97.27% (713/733). The specificity of QFT was 99.06% in the US, 98.76% in Japan, and 95.98% in Australia. The overall specificity of QFT-Plus was 98.09% (719/733).

A breakdown of the results by TB antigen tube type and combinations thereof is shown in Figure 4 to provide an example of expected results in a low-risk population.

Interpretation based on TB Antigen-Nil IU/ml in	TB1	TB2	QFT-Plus (positive by TB1 and/or TB2)*	Concordant positive TB1 and TB2 (alternate analysis)†
Positive	10	18	20	8
Negative	723	715	713	725
Indeterminate	0	0	0	0
Specificity (95% CI)	–	–	97.3% (713/733) (95.8–98.2)	–
Negativity rate (95% CI)	98.6% (723/733) (97.5–99.3)	97.5% (715/733) (96.2–98.4)	–	98.9% (725/733) (97.9–99.5)

* Interpretation based on a TB antigen – Nil value ≥0.35 IU/ml in both (TB1 and TB2) or either TB tube to fit the interpretation criteria for the QFT-Plus (TB1 or TB2) to be determined positive.

† Alternate analysis provided for information only.

Figure 4. Specificity of QFT-Plus by each TB Antigen Tube.

In subjects with low risk for TB infection, 20 of 733 subjects had a positive result. Of these, only 8 subjects returned a value of > 0.35 IU/ml in both TB1 and TB2 tubes.

A comparison of the QFT versus QFT-Plus assays was performed in the low-risk study cohort showed overall concordance of 97.5% (715/733), and a negative percent agreement of 98.3% (707/719).

Clinical Sensitivity

While there is no definitive standard test for LTBI, a surrogate is microbiological culture of M. tuberculosis because TB infection is a necessary precursor to disease.

A multi-center study evaluating the clinical sensitivity of the QFT-Plus was performed in 434 study subjects who presented with signs and symptoms of active M. tuberculosis disease confirmed by culture and/or PCR and were on no TB treatment or within ≤ 14 days of treatment prior to blood collection. The study was performed at 7 independent sites, including 3 in the US, 3 in Japan, and 1 in Australia. QFT-Plus was compared to GIT.
A summary of the clinical sensitivity performance data, stratified by study site and country, is provided in Figure 5. The performance

results are based on the total number of valid tests. The frequency of indeterminate results for GIT and QFT-Plus was 2.3% (10/434) and 2.5% (11/434), respectively.

		Positive		Negative		Indeterminate		Sensitivity (n/N) (95% CI)	
Site	N	QFT	QFT-Plus	QFT	QFT-Plus	QFT	QFT-Plus	QFT	QFT-Plus
United States									
(#1) USA-5	15	13	13	2	2	0	0	86.67% (13/15) (62.12–96.26)	86.67% (13/15) (62.12–96.26)
(#2) USA-1	33	29	29	4	4	0	0	87.88% (29/33) (72.67–95.18)	87.88% (29/33) (72.67–95.18)
(#3) USA-4	5	5	5	0	0	0	0	100.0% (5/5) (56.55–100.0)	100.0% (5/5) (56.55–100.0)
Total United States	53	47	47	6	6	0	0	88.7% (47/53) (77.4–94.7)	88.7% (47/53) (77.4–94.7)
Japan									
(#4) JPN-2	76	72	67	1	3	3	6	98.63% (72/73) (92.64–99.76)	95.71% (67/70) (88.14–98.53)
(#5) JPN-3	99	97	98	2	1	0	0	97.98% (97/99) (92.93–99.44)	98.99% (98/99) (94.50–99.82)
(#6) JPN-1	177	159	157	12	15	6	5	92.98% (159/171) (88.14–95.94)	91.28% (157/172) (86.11–94.64)
Total Japan	352	328	322	15	19	9	11	95.63% (328/343) (92.91–97.33)	94.43% (322/341) (91.5–96.4)
Australia									
(#7) AU-2	29	27	29	1	0	1	0	96.43% (27/28) (82.29–99.37)	100.0% (29/29) (88.30–100.0)

Figure 5. Clinical sensitivity study performance summary stratified by site, country, and overall

Note that the analysis in Figure 5 does not include indeterminate results.

The sensitivity of QFT-Plus was 88.7% in the US, 94.43% in Japan, and 100.0% in Australia. The overall sensitivity of QFT-Plus was 94.09% (398/423). The sensitivity of QFT was 88.7% in the US, 95.63% in Japan, and 96.43% in Australia. The overall sensitivity of QFT was 94.81% (402/424).

A breakdown of the results by TB antigen tube type and combinations thereof is shown in Figure 6 to provide an example of expected results in a population with confirmed TB infection.

Interpretation based on TB Antigen-Nil IU/ml in	TB1	TB2	QFT-Plus (positive by TB1 and/or TB2)
Positive	388	397	398
Negative	32	26	25
Indeterminate	14	11	11
Sensitivity* (95% CI)	–	–	94% (398/423) (91.4–96.0)
Positivity rate* (95% CI)	92.4% (388/420) (89.4–94.6)	93.9% (397/423) (91.1–95.8)	–

* Excluding indeterminate values.

Figure 6. QFT-Plus sensitivity study results by TB antigen tube.

A comparison of GIT and QFT-Plus in a culture-confirmed active TB cohort (sensitivity study cohorts) showed an overall concordance of 95.9% and a positive percent agreement of 97.3% (391/402).

Performance in subjects with identified risk factors for MTB infection (mixed-risk individuals)

A cohort of 601 individuals with mixed risk factors for TB infection (e.g., HIV positivity, history of treatment for active or latent TB, exposure to active TB case, HCW status, etc.) was assessed with both the QFT-GIT (=QFT) and QFT-Plus tests. Risk factors were identified using a standardized survey and individuals displayed no symptoms associated with active TB at the time of recruitment. Demographics and risk factors are reported in Figure 7.

Total subjects (601)		Number	Percentage
Gender	Male	539	89.7%
	Female	62	10.3%
Age (years)	Range	18–70	–
	Mean	46.7	
BCG vaccinated	Yes	15	2.5%
	No	586	97.5%
HIV positive or tested positive for HTLV viruses	Yes	12	2.0%
	No	589	98%
Previously diagnosed with active TB	Yes	11	1.8%
	No	590	98.2%
Had a positive Tuberculin Skin Test (TST)/Mantoux test for TB	Yes	47	7.8%
	No	554	92.2%
Ever been treated for active or latent TB	Yes	35	5.8%
	No	566	94.2%
Lived, worked or volunteered (>1 month) in a jail or prison	Yes	373	62.1%
	No	228	37.9%
Lived, worked or volunteered (>1 month) in a homeless shelter	Yes	525	87.4%
	No	76	12.6%
Healthcare worker	Yes	8	1.3%
	No	593	98.7%
Close contact of someone with or suspected of having active TB disease	Yes	9	1.5%
	No	592	98.5%

Figure 7. Demographics and factors associated with risk of TB infection in a mixed cohort.

In this population, 68/601 (11.3%) subjects generated a positive QFT-Plus result. Of the 68 QFT-Plus positive subjects, a total of 62 subjects were positive by both TB1 and TB2 tubes, 2 subjects were positive by TB1 only, and 4 subjects were positive by TB2 only. No indeterminate results (0/601) were observed.

		QFT		
		Positive (+)	Negative (–)	Total
QFT-Plus	Positive (+)	63	5*	68
	Negative (–)	1*	532	533
	Total	64	537	601

*All 6 discordant samples had IFN-γ levels of the TB Antigen tubes that were close to the assay cut-off.

Figure 8. Performance summary: QFT-Plus versus QFT in subjects with known risk factors for LTBI.

The positive percent agreement and negative percent agreement between between QFT and QFT-Plus were as follows:

- PPA: 98.44% (63/64), 95%CI (91.67, 99.72)
- NPA: 99.07% (532/537), 95% CI (97.84, 99.60)

Figure 8 illustrates the performance of QFT-Plus compared to QFT in BCG vaccinated study subjects.

		QFT		
		Positive (+)	Negative (–)	Total
QFT-Plus	Positive (+)	66	5	71
	Negative (–)	3	268	271
	Total	69	273	342*

*Two Sensitivity Study Subjects were excluded from the analysis due to indeterminate results.

Figure 9. Performance of QFT-Plus compared to QFT in BCG-vaccinated study subjects (combined data from sensitivity, specificity, and LTBI study subjects).

The resulting PPA and NPA are as follows:
PPA: 95.6% (66/69), 95%CI (87.98, 98.51)

- NPA: 98.2% (268/273), 95%CI (95.79, 99.22)

Clinical performance was demonstrated based on a systematic literature review, clinical performance studies with clinical performance indicators such as sensitivity, specificity**,** positive percent agreement (PPA), negative percent agreement (NPA), concordance with other IGRAs, and (published) experience gained by routine diagnostic testing. The assessment of these sources showed that the

	clinical performance of the QFT-Plus test is adequate for its Intended Use.
6.4 Summary of performance data from other sources, if applicable	Not applicable
6.5 An overall summary of the performance and safety	In regards to safety, the overall benefit-risk assessment based on systematic literature and database review, risk assessment activities (medical risk assessment, manufacturing and user risk assessments), vigilance activities conducted by QIAGEN, and experience gained from routine diagnostic testing supports a favourable benefit-risk ratio for the QFT-Plus test and is adequate in reference to the current state of the art.
6.6 Ongoing or planned post-market performance follow-up	Based on the density and validity of available analytical and clinical data, there are currently no open questions for QFT-Plus. Collected evidence which shows that the QFT-Plus Test meets the performance evaluation requirements the assay is considered safe and effective for its intended use and no acceptable residual risks remain, it was concluded that no PMPF activities are currently required for this device. QIAGEN has implemented and maintains surveillance programs that routinely monitor the clinical performance and safety of the product. This includes proactive collection and evaluation of safety, performance, scientific data, and re-assessment of benefit–risk ratio. Post-market data are gathered from a variety of sources, such as clinical experience of the device in routine use, feedback from users/distributors/importers, trending, screening of relevant published technical and scientific literature or data on quality. In addition, safety and adverse event reports are evaluated.
7. Metrological traceability of assigned values	
7.1 Explanation of the unit of measurement, if applicable	The information and instructions provided by the manufacturer are easy for the intended user to understand and apply, to correctly interpret the result provided by the device and to avoid misleading information.

	QFT-Plus Analysis Software can be used to analyze raw data and calculate results. It is available at www.QuantiFERON.com. Please make sure that the latest version of the QFT-Plus 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. The software reports all concentrations greater than 10 IU/ml as ">10" as such values fall beyond the validated linear range of the ELISA. As an alternative to using the QFT-Plus Analysis Software, results can be determined according to the following method. <u>Generation of standard curve and sample values</u> If QFT-Plus Analysis Software is not used Determination of the standard curve and determination of sample IU/ml values require a spreadsheet program, such as Microsoft® Excel®, if the QFT-Plus software is not used. Using a spreadsheet program: 1. Determine the mean OD values of the kit standard replicates on each plate. 2. 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. 3. 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. 4. 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. IFN-γ values (in IU/ml) for the TB1, TB2, and Mitogen tubes are corrected for background by subtracting the IU/ml value obtained for the respective Nil control. These corrected values are used for interpretation of the test results. <u>Quality control of the test</u> 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 values must be ≤ 15%. • Replicate OD values for Standard 3 and Standard 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. • 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. The QFT-Plus Analysis Software calculates and reports these quality control parameters.
7.2 Identification of applied reference materials and/or reference measurement procedures of higher order used by the	The QFT-Plus ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535).

manufacturer for the calibration of the device	
8. Suggested profile and training for users	
8.1 Suggested profile and training for users	This kit is intended for professional use. The product is to be used only by personnel specifically instructed and trained in good laboratory practices techniques and who are familiar with this technology. The product is to be used only by personnel specifically instructed and trained in good laboratory practices techniques and who have been trained to perform this assay.

Revision History

SSP Revision Number	Date issued	Change description	Revision validated by the Notified Body
01	February 2023	Generation of document	☒ Yes Validation Language: English □ No (only applicable for class C (IVDR, Article 48 (7)) for which the SSP is not yet validated by the NB)
02	February 2024	Transfer to a new template according to MDCG 2022-9	☒ Yes Validation Language: English □ No (only applicable for class C (IVDR, Article 48 (7)) for which the SSP is not yet validated by the NB)