SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Test, Immunity, Cell mediated, Mycobacterium tuberculosis
Device Trade Name:	LIAISON QuantiFERON-TB Gold Plus, LIAISON Control QuantiFERON-TB Gold Plus, LIAISON QuantiFERON Software
Device Procode:	NCD
Applicant's Name and Address:	DiaSorin Inc. 1951 Northwestern Avenue Stillwater, MN 55082-0285
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P180047

Date of FDA Notice of Approval: November 26, 2019

II. INDICATIONS FOR USE

The **LIAISON QuantiFERON-TB Gold Plus** assay is an *in vitro* diagnostic test for the detection of interferon-γ (IFN-γ) in human lithium heparin plasma by chemiluminescence immunoassay (CLIA) using the LIAISON XL Analyzer. QIAGEN QuantiFERON-TB Gold Plus Blood Collection Tubes, containing a peptide cocktail simulating ESAT-6, and CFP-10 proteins, are used in conjunction with the LIAISON QuantiFERON-TB Gold Plus assay to stimulate cells in heparinized whole blood. Detection of IFN-γ is used to identify *in vitro* responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

The assay is a qualitative 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 to assist the clinician in making individual patient management decisions. The LIAISON QuantiFERON-TB Gold Plus assay must be performed using the LIAISON XL Analyzer.

The **LIAISON Control QuantiFERON-TB Gold Plus** is intended for use as assayed quality control samples to monitor the performance of the LIAISON QuantiFERON-TB Gold Plus assay. The performance characteristics of LIAISON Control QuantiFERON-TB Gold Plus have not been established for any other assays or instrument platforms other than the LIAISON XL Analyzer.

The **LIAISON QuantiFERON Software (LQS)** is optional software intended to analyze the data generated by the LIAISON QuantiFERON-TB Gold Plus assay on the LIAISON XL Analyzer. LQS reports assay results as positive, negative, or indeterminate by an algorithm that combines the individual results associated with the four QIAGEN QuantiFERON-TB Gold Plus Blood Collection Tubes into a final result.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions specific to the LIAISON QuantiFERON-TB Gold Plus assay, the LIAISON Control QuantiFERON-TB Gold Plus, and the LIAISON QuantiFERON Software (LQS), can be found in the respective package insert labeling.

V. DEVICE DESCRIPTION

The LIAISON QuantiFERON-TB Gold Plus [an interferon-γ release assay IGRA)] is a qualitative chemiluminescence immunoassay assay performed on the LIAISON XL Analyzer designed to be used in conjunction with QuantiFERON-TB Gold Plus (QFT-Plus) Blood Collection Tubes which are manufactured by QIAGEN Sciences LLC[1]. The four QFT-Plus Blood Collection Tubes are used for the collection and stimulation of whole blood samples and are an essential component of the LIAISON QuantiFERON-TB Gold Plus assay.

1) QFT-Plus TB1 and TB2 Blood Collection Tubes contain a peptide cocktail simulating ESAT-6 and CFP-10 proteins of *Mycobacterium tuberculosis* that elicit IFN-γ production.

2) QFT-Plus Mitogen and Nil Blood Collection Tubes serve as assay positive and negative controls, respectively. The QFT-Plus Mitogen tube contains phytohemagglutinin that stimulates T-cells and the production of IFN-γ. The QFT-Plus Nil tube contains no antigens and is used to adjust for pre-existing IFN-γ (i.e., The IFN-γ level of the Nil tube sample is subtracted from the IFN-γ levels of the TB1, TB2, and Mitogen tube samples).

Each whole blood sample collected using the four QFT-Plus Blood Collection Tubes is handled and processed in accordance with *QFT-Plus Specimen Collection and Handling* procedures in the QFT-Plus Package Insert. The resulting plasma samples are transferred to the LIAISON XL[2] Analyzer for detection of the analyte IFN-γ.

[1] QIAGEN Sciences LLC is the contract manufacturer of the QFT-Plus Blood Collection Tubes. The QFT-Plus Blood Collection Tubes are a critical component of the LIAISON QuantiFERON-TB Gold Plus assay.
[2] The LIAISON XL Analyzer instrument and software were reviewed and cleared as part of 510(k) K181464.

The LIAISON XL Analyzer, controlled by LIAISON XL Software, is fully automated and performs sample processing (sample pre-dilutions, sample and reagent dispensing, incubations, wash processes) as well as measurement and evaluation.

The LIAISON QuantiFERON-TB Gold Plus detects the analyte IFN-γ by direct sandwich chemiluminescence immunoassay (CLIA). During the first incubation, anti-IFN-γ monoclonal (mouse) antibodies with solid phase magnetic particles, and anti-IFN-γ monoclonal (mouse) antibodies conjugated with isoluminol, will bind to any IFN-γ present in the test sample (or controls, or calibrators) and form a sandwich. Any unbound material in the sample is removed with a wash cycle. During the second incubation, Assay Buffer W is added to reduce sample related non-specific binding, followed by a second wash cycle. Next the starter reagents of the LIAISON XL Analyzer are added and a flash chemiluminescent reaction is induced. The light signal which reflects the amount of isoluminol-antibody conjugate (indicative of the amount of IFN-γ in the test sample, control, or calibrator), is measured by a photomultiplier as relative light units (RLUs). The RLUs are evaluated and translated to amount of IFN-γ is generated, in International Units (IU)/mL.

The LIAISON QuantiFERON-TB Gold Plus assay results are interpreted via the use of an algorithm (Table 1) that combines the results from each of the four QFT-Plus Blood Collection Tubes. The final assay result is qualitative (i.e., positive, negative, indeterminate).

The LIAISON QuantiFERON Software (LQS) is optional software that may be used to assist the user with assay data analysis and results interpretation. The LQS calculates results as positive, negative, or indeterminate, based on an algorithm that combines the individual results associated with the four QIAGEN QuantiFERON-TB Gold Plus Blood Collection Tubes.

Table 1. LIAISON QuantiFERON-TB Gold Plus Assay Results Interpretation Algorithm

<table>
<tr><th>Nil (IU/ml)</th><th>TB1 minus Nil (IU/ml)</th><th>TB2 minus Nil (IU/ml)</th><th>Mitogen minus Nil (IU/ml)</th><th>LIAISON QuantiFERON - TB Gold Plus result</th><th>Report/ Interpretation</th></tr>
<tr><td rowspan="4">≤8.0</td><td>≥0.35 and ≥ 25% of Nil</td><td>Any</td><td rowspan="2">Any</td><td rowspan="2">Positive</td><td rowspan="2">M. tuberculosis infection likely</td></tr>
<tr><td>Any</td><td>≥0.35 and ≥ 25% of Nil</td></tr>
<tr><td><0.35 OR ≥0.35 and < 25% of Nil</td><td><0.35 OR ≥0.35 and < 25% of Nil</td><td>≥0.5</td><td>Negative</td><td>M. tuberculosis infection NOT likely</td></tr>
<tr><td><0.35 OR ≥0.35 and < 25% of Nil</td><td><0.35 OR ≥0.35 and < 25% of Nil</td><td><0.5</td><td rowspan="2">Indeterminate</td><td rowspan="2">Likelihood of M.tuberculosis infection cannot be determined</td></tr>
<tr><td>>8.0</td><td colspan="3">Any</td></tr>
</table>

Components of the LIAISON QuantiFERON-TB Gold Plus Assay

The LIAISON QuantiFERON-TB Gold Plus assay kit (200 tests) consists of three reagents (Magnetic particles, Diluent, Assay Buffer W) provided in individual compartments within a plastic container called a Reagent Integral, and four additional reagents (Calibrator A, Calibrator B, Buffer R, and Conjugate) are provided in individual glass vials. The contents of each reagent are further described below:

Reagent Integral:

a. MAGNETIC PARTICLES – Magnetic particles coated with mouse anti-human IFN-γ monoclonal antibody, BSA, phosphate buffer, < 0.1% sodium azide. One vial, total volume is 2.5 mL. Ready to use.
b. DILUENT- BSA, casein, phosphate buffer, EDTA, 0.2% ProClin 300, Polyclonal Mouse Nonspecific IgG, gentamycin sulfate 0.1 g/L. One vial, total volume is 18 mL. Ready to use.
c. ASSAY BUFFER W – BSA, casein, phosphate buffer, EDTA, 0.2% ProClin 300, and an inert blue dye. Two vials, volume of each vial is 23 mL. Ready to use.

Individual Reagent Vials:

d. CALIBRATOR A – Recombinant human IFN-γ (produced in E.coli), HEPES buffer, BSA, bovine serum, 0.4% ProClin 300, 0.2 µg/L gentamycin sulfate, detergents. One vial, lyophilized. Total volume once reconstituted is 2.0 mL.
e. CALIBRATOR B – Recombinant human IFN-γ (produced in E.coli), HEPES buffer, BSA, bovine serum, 0.4% ProClin 300, 0.2 µg/L gentamycin sulfate, detergents. One vial, lyophilized. Total volume once reconstituted is 2.0 mL
f. BUFFER R – Streptavidin conjugated with isoluminol derivative, BSA, casein, phosphate buffer, 0.2% ProClin 300, gentamycin sulphate 0.1 g/L, Polyclonal Mouse Nonspecific IgG, detergents. Two vials, volume of each vial is 4.5 mL. Ready to use.
g. CONJUGATE – Biotinylated mouse anti-human IFN-γ monoclonal antibody, HEPES buffer, BSA, casein, Polyclonal Mouse Nonspecific IgG, 0.2% ProClin 300, gentamycin sulphate 0.1 g/L, detergents, anti-proteases. Two vials, lyophilized. Volume of each vial once reconstituted is 4 mL.

Components of the LIAISON Control QuantiFERON-TB Gold kit:

LIAISON Control QuantiFERON-TB Gold Plus kit is an additional material required to perform LIAISON QuantiFERON-TB Gold Plus assay, and is used for monitoring assay performance. Each kit consists of two controls, each with a specific concentration that is predetermined to be within a defined range of analyte (IFN-γ) concentration. The analyte concentration is established by DiaSorin (the manufacturer of the controls), and is described in the Certificate of Analysis (CoA). Two vials of each control are provided lyophilized with each kit. Each control vial contains enough reagent for 20 tests. The contents of each of the controls are further described below:

a. Control 1 – Recombinant human IFN-γ (produced by *E.coli*), HEPES buffer, BSA, bovine serum, 0.4% ProClin 300, 0.2 g/L gentamycin sulfate. Total volume of each vial once reconstituted is 2 mL.
b. Control 2 - Recombinant human IFN-γ (produced by *E.coli*), HEPES buffer, BSA, bovine serum, 0.4% ProClin 300, 0.2 g/L gentamycin sulfate. Total volume of each vial once reconstituted is 2 mL.

Additional Components Required by Not Provided in the LIAISON QuantiFERON-TB Gold Plus assay kit.

LIAISON XL Analyzer
LIAISON XL Cuvettes (REF X0016)
LIAISON XL Disposable Tips (REF X0015)
LIAISON XL Starter Kit (REF 319200)
LIAISON XL Wash/System Liquid (REF 319100)
LIAISON XL Waste Bags (REF X0025)

Required Materials from Other Supplier
QIAGEN QuantiFERON-TB Gold Plus Blood Collection Tubes:
- #622536 – 200 Count (50 each of Nil, TB1, TB2, and Mitogen tube)
- #622433 – 25 Dispenser Packs per carton (each pack includes 1 Nil, 1 TB1, 1 TB2, and 1 Mitogen tube)
- #623536 – High Altitude 200 Count (50 each of Nil, TB1, TB2, and Mitogen tube)

Quality Control Procedures

a. Assay Calibration - LIAISON QuantiFERON-TB Gold Plus Assay

Assay calibration must be tested and validated using two kit calibrators (Calibrator A, Calibrator B), to compensate for variability in assay reagent kit lots, LIAISON XL analyzers, and environmental conditions. The results from the calibrators are used to establish working curves when compared to assay master curves stored in the LIAISON XL analyzer. More specifically, the stored master curve is generally defined with 10 master curve base points. The two assay kit calibrators with defined analyte concentrations are measured. The measurement signals [in relative light units (RLU)] of both calibrators are compared to the master curve signals of the corresponding calibrator concentrations. The measurement signals of the calibrators allow the shift of all master curve points to a working curve, corresponding with the actual conditions during measurement. Calibration is required every 4 weeks or when quality control test results fall outside of acceptable limits. In addition, calibration is required when a new reagent lot is tested or after servicing of the LIAISON XL analyzer.

b. Quality Control (QC) - LIAISON Control QuantiFERON-TB Gold Plus

A set of assay controls must be tested to monitor the reagents and performance of the LIAISON QuantiFERON-TB Gold Plus assay. The analyte concentration of each control

indicates the limits established by DiaSorin for control values that can be obtained in reliable assay test runs, and is indicated on the accompanying Certificate of Analysis (CoA). QC testing should be performed at least once per day of use, or according to guidelines or requirements of accredited organizations or local regulations. When QC test results fall outside the expected range of analyte concentration, calibration should be repeated and controls and samples retested. Patient sample results should not be reported until QC test results fall within the analyte expected range. Once reconstituted, assay controls are stable for four weeks, when stored at 2-8°C.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are two FDA approved IVD IFN-γ release assays currently on the market. Similar to the LIAISON QuantiFERON-TB Gold Plus assay, these devices are qualitative indirect tests intended for use in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations to aid the diagnosis of *Mycobacterium tuberculosis* infection (including disease).

VII. MARKETING HISTORY

The LIAISON QuantiFERON-TB Gold Plus assay and LIAISON Control QuantiFERON-TB Gold (CE-marked products) are currently marketed in multiple countries. These devices have not been withdrawn from the market in any country to date for any reasons related to safety and effectiveness. A list of countries where the CE-marked products are currently marketed is illustrated in Table 2 below.

Table 2. Countries Currently Marketing CE-marked LIAISON QuantiFERON-TB Gold Plus assay and LIAISON Control QuantiFERON-TB Gold

• Austria	• Norway
• Belgium	• Portugal
• Denmark	• Spain
• Finland	• Sweden
• France	• Switzerland
• Germany	• United Kingdom
• Italy	
• Luxembourg	
• Netherlands	

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, the LIAISON QuantiFERON-TB Gold Plus poses no known major direct adverse effects to health.

Minor adverse reactions may occur during blood collection associated with venipuncture to include pain and/or redness at the site of venipuncture, slight risk of bleeding,

hematoma, and skin infection. Other mild reactions associated with venipuncture may include: agitation, sweating, pallor, coldness, sense of weakness, nausea, or fainting.

Failure of the product to perform as intended, or incorrect performance of the assay, may lead to false positive or false negative results. The risk of false positive or false negative results negatively impacting the determination of infection with *M. tuberculosis*, and subsequent patient care, is mitigated by the requirement that all LIAISON QuantiFERON-TB Gold Plus assay results be interpreted in conjunction with patient risk factors, radiography, and other medical and diagnostic evaluations.

IX. SUMMARY OF NONCLINICAL STUDIES

A. 20-Day Precision Study

A precision study was conducted at DiaSorin S.p.A. in Italy. A coded test panel of 10 contrived samples was prepared by spiking analyte negative lithium heparinized plasma samples with native IFN-γ. Native IFN-γ was obtained from plasma harvested after incubation/stimulation of whole blood in QFT-Plus Mitogen blood collection tubes. The 10-member test panel was prepared with established IFN-γ concentrations that spanned the LIAISON QuantiFERON-TB Gold Plus assay result range (Table 3). The 10-member sample panel was tested along with the two LIAISON QuantiFERON-TB Gold controls (one kit lot). Testing was conducted in two runs per day, two replicates per run, using two LIAISON QuantiFERON-TB Gold Plus kit lots (#291001, and #291003) for a total of 160 test results per sample member. Study testing was conducted by a total of three operators and spanned two calibration cycles.

Table 3. 20-Day Precision Study Sample Test Panel

PANEL	Sample ID	Classification IU/mL
PRECISION PANEL	QFTB-01-P01	0.000 - 0.350
	QFTB-01-P02	0.000 - 0.350
	QFTB-01-P03	0.350 - 2.00
	QFTB-01-P04	0.350 - 2.00
	QFTB-01-P05	0.350 - 2.00
	QFTB-01-P06	2.00 - 5.00
	QFTB-01-P07	2.00 - 5.00
	QFTB-01-P08	5.00 – 10.0
	QFTB-01-P09	5.00 – 10.0
	QFTB-01-P10	5.00 – 10.0
Kit Control	#7124010	≤ 0.200
	#7125010	0.826 – 2.18

The results obtained are relatively consistent across both LIAISON QuantiFERON-TB Gold Plus kit lots and appear to demonstrate good precision. A summary of the study results is illustrated in Tables 4 and 5 below.

Table 4. 20-Day Precision Study Summary Results #1 - LIAISON QuantiFERON-TB Gold Plus Kit Lots 291001 and 291003

Panel Member	n	Mean IFN-γ IU/mL	Within Lot - #291001		Within Lot - #291003	
			SD	%CV	SD	%CV
QFTB-01-P01	160	0.241	0.021	9.014	0.021	8.449
QFTB-01-P02	160	0.294	0.028	9.679	0.024	8.156
QFTB-01-P03	160	0.525	0.040	7.568	0.038	7.270
QFTB-01-P04	160	0.797	0.065	8.162	0.064	8.059
QFTB-01-P05	160	1.556	0.126	8.030	0.139	9.032
QFTB-01-P06	160	2.988	0.269	8.845	0.263	8.956
QFTB-01-P07	160	3.913	0.367	9.257	0.358	9.257
QFTB-01-P08	160	5.494	0.498	8.920	0.483	8.935
QFTB-01-P09	160	5.851	0.485	8.186	0.542	9.379
QFTB-01-P10	160	6.678	0.603	8.948	0.640	9.686
#7124010 (Control Level 1)	160	0.074	0.018	29.995	0.019	21.134
#7125010 (Control Level 2)	160	1.520	0.122	8.335	0.148	9.442

Table 5. 20-Day Precision Study Summary Results #2 - LIAISON QuantiFERON-TB Gold Plus Kit Lots 29100 and 291003

Panel Member	N	Mean	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Lab (Total)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QFTB-01-P01	160	0.241	0.008	3.121	0.005	2.242	0.019	7.836	0.005	1.932	0.022	8.939
QFTB-01-P02	160	0.294	0.011	3.590	0.007	2.263	0.023	7.854	0.003	1.169	0.026	9.003
QFTB-01-P03	160	0.525	0.012	2.250	0.011	2.076	0.035	6.674	0.000	0.000	0.039	7.343
QFTB-01-P04	160	0.797	0.020	2.514	0.013	1.637	0.059	7.455	0.000	0.000	0.064	8.036
QFTB-01-P05	160	1.556	0.035	2.252	0.033	2.149	0.123	7.899	0.000	0.000	0.132	8.490
QFTB-01-P06	160	2.988	0.090	3.006	0.034	1.125	0.248	8.301	0.039	1.318	0.269	8.997
QFTB-01-P07	160	3.913	0.090	2.308	0.081	2.079	0.340	8.700	0.000	0.000	0.361	9.238
QFTB-01-P08	160	5.494	0.121	2.201	0.093	1.687	0.466	8.487	0.053	0.967	0.493	8.980
QFTB-01-P09	160	5.851	0.121	2.068	0.155	2.644	0.474	8.108	0.000	0.000	0.513	8.775
QFTB-01-P10	160	6.678	0.173	2.585	0.243	3.633	0.542	8.124	0.000	0.000	0.619	9.267
#7124010	160	0.074	0.009	11.943	0.009	11.554	0.013	18.207	0.022	29.419	0.028	38.381
#7125010	160	1.520	0.036	2.345	0.040	2.613	0.125	8.231	0.067	4.398	0.152	9.971

B. Analytical Specificity/Cross-Reactivity

B.1 Study One - Potential Interference/Cross-Reactivity of Endogenous Substances

Study One was performed to evaluate potential LIAISON QuantiFERON-TB Gold Plus assay interference/cross-reactivity due to endogenous substances.

Test samples were prepared using confirmed analyte-negative plasma samples spiked with native IFN-γ. A total of three samples, each prepared at a high non-reactive, around the cutoff (0.35 IU/mL), and low-reactive (0.5-1.0 IU/mL) concentration were used in this study. Each sample was divided into two aliquots, the first aliquot spiked with a volume of

the potential interfering substance to achieve a high concentration, and the second aliquot spiked with the same volume of water to serve as a control. The endogenous potential interfering substances and the concentrations tested are illustrated in Table 6 below. All samples were tested in the same run, in 26 replicates each, using one lot of the LIAISON QuantiFERON-TB Gold Plus assay kit reagents, and one lot of kit controls.

Table 6. Study One - Potential Interfering Endogenous Substances and Concentrations Tested

Substance	Concentrations Tested
IL-2	10 ng/mL
IL-4	5 ng/mL
IL-5	100 ng/mL
IL-6	100 ng/mL
IL-10	100 ng/mL
IL-12	100 ng/mL
IFN- alpha	50 ng/mL
IFN- beta	50 ng/mL
TNF-alpha	5 ng/mL

Test sample results were deemed acceptable if the percent interference fell within ± 10% of that of the control samples. A summary of the study results are illustrated in Table 7 below. Test sample results are depicted to the right of the control sample results.

Table 7. Study One – Interference/Cross-Reactivity of Endogenous Substances Study Summary Results

Sample	High Non-Reactive			Sample around cut off value			Low Reactive		
Substance	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT
IL-2 10 ng/mL	0.260	0.250	3.92	0.642	0.637	0.80	0.969	0.956	-1.35
IL-4 5 ng/mL	0.265	0.267	0.81	0.702	0.696	-0.86	1.03	1.02	-0.28
IL-5 100 ng/ml	0.262	0.267	1.79	0.676	0.706	4.47	1.01	1.01	0.31
IL-6 100 ng/mL	0.256	0.259	1.34	0.626	0.641	2.41	0.993	0.993	-0.03
IL-10 100 ng/mL	0.257	0.267	4.01	0.602	0.612	1.55	0.951	1.01	6.10
IL-12 100 ng/mL	0.252	0.258	2.52	0.621	0.631	1.64	0.997	0.954	-4.00
IFN- alpha 50 ng/mL	0.216	0.210	-2.49	0.545	0.538	-1.19	0.831	0.867	4.38
IFN- beta 50 ng/mL	0.215	0.223	3.96	0.547	0.520	-5.00	0.859	0.891	3.79
TNF-alpha 5 ng/mL	0.256	0.251	-1.80	0.589	0.598	1.66	0.974	0.960	-1.46

B.2 Study Two - Potential Interference/Cross-reactivity of Endogenous and Pharmacological Substances

A second interference study (Study Two) was performed to evaluate the potential interference of endogenous and pharmacological substances at concentrations listed in Table 8 below. Study Two employed the same study design and acceptance criteria as Study One.

Table 8. Study Two - Potential Interfering Endogenous and Pharmacological Substances and Concentrations Tested

Substance	Kind of substance	Normal range	Reference for test concentration	Concentration to be tested	Vehicle
Triglycerides	**Endogenous**	**0.34 – 3.7 mmol/L**	**EP7-A2**	**3000 mg/dL**	**Plasma**
Hemoglobin	**Endogenous**	**1 – 2 g/L**	**EP7-A2**	**1000 mg/dL**	**Plasma**
Unconjugated bilirubin	**Endogenous**	**5 - 21 µmol/L**	**EP7-A2**	**20 mg/dL**	**H_2O**
Conjugated bilirubin	**Endogenous**	**0.0 - 0.34 mmol/L**	**EP7-A2**	**20 mg/dL**	**H_2O**
Cholesterol	**Endogenous**	**2.95 -5.2 mmol/L**	**EP7-A2**	**350 mg/dL**	**Plasma**
Prednisolone	**Pharmacological**	**Not applicable**	**EP7-A2-Appendix C**	**0.3 mg/dL**	**H_2O**
Cyclosporine	**Pharmacological**	**Not applicable**	**Information provided by Qiagen**	**5 µg/mL**	**H_2O**
Abacavir sulfate	**Pharmacological**	**Not applicable**	**Information provided by Qiagen**	**15 µg/mL**	**H_2O**
Biotin	**Pharmacological**	**Not applicable**	**Ref. L-13-10-064-M**	**3500 ng/mL**	**H_2O**

A summary of the study results are illustrated in Table 9 below. Test sample results are depicted to the right of the control sample results.

Table 9. Study Two – Interference/Cross-Reactivity of Endogenous and Pharmacological Substances Study Summary Results

Sample	High Non-Reactive			Sample around cut off value			Low Reactive		
Substance	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT
Triglycerides 3000 mg/dL	0.217	0.211	-3.05	0.569	0.565	-0.70	1.18	1.20	1.37
Hemoglobin 1000 mg/dL	0.231	0.233	0.63	0.600	0.616	2.80	0.963	0.949	-1.50
Unconjugated bilirubin 20 mg/dL	0.225	0.230	2.55	0.586	0.592	1.04	0.916	0.911	-0.49
Conjugated bilirubin 20 mg/dL	0.225	0.227	1.11	0.586	0.590	0.68	0.916	0.879	-3.99
Cholesterol 350 mg/dL	0.217	0.218	0.18	0.569	0.581	2.08	1.18	1.22	3.13
Prednisolone 0.3 mg/dL	0.197	0.198	0.86	0.542	0.546	0.76	0.892	0.899	0.73
Cyclosporine 5 µg /mL	0.192	0.196	1.84	0.489	0.469	-4.04	0.782	0.795	1.67
Abacavir sulfate 15 µg/mL	0.225	0.231	2.43	0.557	0.525	-5.73	0.927	0.928	0.14
Biotin 3500 ng/mL	0.227	0.230	1.54	0.554	0.558	0.66	0.915	0.924	1.01

B.3. Study Three - Potential Interference/Cross-Reactivity of Total Protein, Rheumatoid Factor, and Human Anti-Murine Antibody (HAMA).

A third interference study (Study Three) was performed to evaluate the potential interference of total protein, rheumatoid factor, and HAMA. Study Three employed the same study design and acceptance criteria as Study One. Each potential interferent and associated concentrations tested are listed in Table 10 below.

Table 10. Study Three – Potential Interferents – Total Protein, Rheumatoid Factor, HAMA, and Concentrations Tested

Substance	Normal range	Reference for test concentration	Concentration to be tested
Total protein (high)	60 – 80 g/L	EP7-A2	120 g/L
Total protein (low)	60 – 80 g/L	EP7-A2	< 60 g/L
Rheumatoid Factor	No reference level		The highest RF concentration possible
HAMA	No reference level		The highest HAMA concentration possible

A summary of the study results are illustrated in Table 11 below. Test sample results are depicted to the right of the control sample results.

Table 11. Study Three – Interference/Cross-Reactivity of Total Protein, Rheumatoid Factor, and HAMA Study Summary Results

Sample	High Non-Reactive			Sample around cut off value			Low Reactive		
Substance	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT	Mean IU/mL Control	Mean IU/mL	% INT
Total protein (high) 120 g/L	0.340	0.329	-3.2	0.499	0.477	-4.4	0.964	0.948	-1.6
Total protein (low) 38 g/L	0.340	0.341	0.27	0.499	0.535	7.1	0.964	0.986	2.4
Rheumatoid Factor 469 IU/mL	0.320	0.306	-4.31	0.458	0.484	5.71	0.919	0.925	0.57
HAMA 600 ng/mL	0.325	0.315	-2.95	0.524	0.486	-7.27	0.918	0.889	-3.16

With regard to all three interference/cross-reactivity studies, the concentrations of all potential interfering substances appear to provide sufficient challenge to the device, and the study results suggest the performance of the LIAISON QuantiFERON-TB Gold Plus assay is not affected by elevated levels of the substances evaluated.

C. Limit of Blank (LoB)

A study was conducted to determine the Limit of Blank (LoB) of the LIAISON QuantiFERON-TB Gold Plus assay. The aim of the study was to determine the highest value (IU/mL) expected of a natural plasma sample that is confirmed analyte (IFN-γ) negative. The study was conducted using a test sample panel that consisted of five lithium heparin plasma samples, confirmed to be analyte negative. Testing was performed in duplicate on two different LIAISON XL Analyzer instruments. Testing was conducted using two assay kit lots and one control kit lot; one instrument and one operator per assay kit lot, and six test runs conducted over three days. A total of 60 blank results were obtained for each assay kit lot. The LoB of each assay kit lot tested was calculated using the following formula:

LoB = (Mean of All Blank Samples tested) + 1.653(Standard Deviation of All Blank Samples tested)[3]

The LoB acceptance criterion was defined as the highest LoB value among product lots/instruments/operators. A summary of the LoB study results is illustrated in Table 12 below.

[3] LoB formula calculation is consistent with CLSI guideline EP-17 A2, *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -Second Edition Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -Second Edition*.

Table 12. Limit of Blank (LoB) Study Summary Results

LIAISON QuantiFERON-TB Gold Plus assay Kit Lot	#291006	#291007
LIAISON XL Analyzer Instrument No.	2206000004	2206000003
Blank Dose Mean (IU/mL)	0.032	0.035
Blank Dose SD (IU/mL)	0.020	0.010
Calculated LoB (IU/mL)	0.065	0.052

The highest LoB value obtained was 0.065 IU/mL, and is therefore determined to be the established LoB for the LIAISON QuantiFERON-TB Gold Plus assay.

D. High Dose Hook Effect

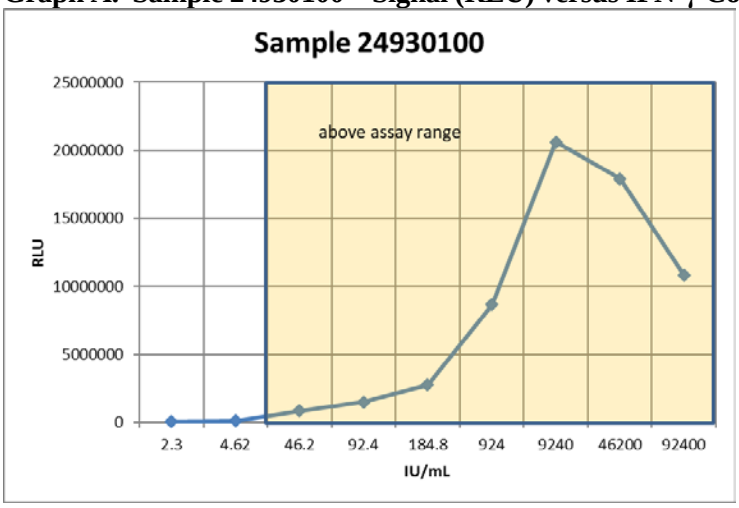
A study was conducted to assess the saturation effect that may occur when testing samples containing very high levels of analyte, resulting in a decrease in the actual analyte concentration detected. Test samples were prepared by spiking confirmed analyte negative lithium heparin plasma with elevated concentrations of IFN-γ (either native or recombinant). The study summary results were illustrated in Table 13 and Graphs A, B, and C, below.

Table 13. High Dose Hook Effect Study Summary Results

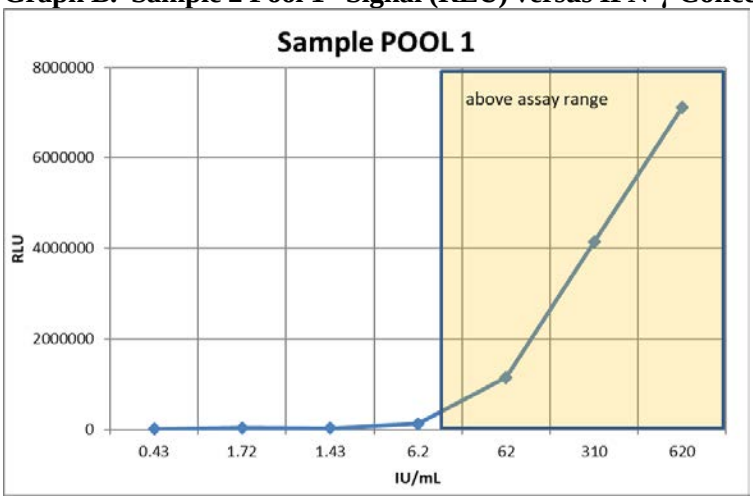
	Sample 24930100			Sample 2 Pool 1			Sample 3 Pool 2		
Dilution factor (1/x)	Mean RLU	Mean IU/mL	IU/mL (based on dilution factor applied)	Mean RLU	Mean IU/mL	IU/mL (based on dilution factor applied)	Mean RLU	Mean IU/mL	IU/mL (based on dilution factor applied)
NEAT	10834455	>10	92400	7115561	>10.0	620	7819727	>10.0	566
1:2	17916338	>10	46200	4145415	>10.0	310	4269435	>10.0	283
1:10	20624777	>10	9240	1151196	>10.0	62.0	1144409	>10.0	56.6
1:100	8667421	>10	924	127539	6.20	6.20	116160	5.66	5.66
1:500	2754273	>10	185	28685	1.43	1.43	26937	1.34	1.34
1:1000	1490507	>10	92.4	34329	1.72	1.72	14375	0.726	0.726
1:2000	838296	>10	46.2	8418	0.430	0.430	7214	0.364	0.364
1:20000	93046	4.62	4.62	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1:40000	46157	2.30	2.30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Sample 24930100 = Test sample prepared by spiking confirmed analyte negative human plasma with commercially available recombinant human IFN-γ.
Sample 2 Pool 1 and Sample 3 Pool 2 = Test sample prepared by spiking confirmed analyte negative human plasma with pooled residual native human IFN-γ.

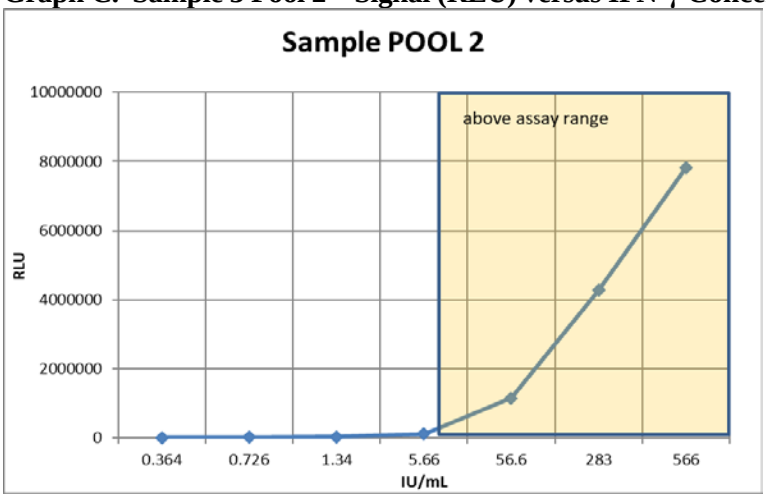
Graph A. Sample 24930100 – Signal (RLU) versus IFN-γ Concentration



Graph B. Sample 2 Pool 1– Signal (RLU) versus IFN-γ Concentration



Graph C. Sample 3 Pool 2 – Signal (RLU) versus IFN-γ Concentration



The study demonstrates that no hook effect was observed until IFN-γ levels reached 9240 IU/mL. However samples with an analyte level value up to 92,400 IU/mL still resulted in a signal above the maximum range of > 10 IU/mL.

E. Analyte Carry-Over Study

The LIAISON XL Analyzer has one dedicated sample dispense needle, one dedicated reagent needle, and utilizes disposable tips for both sample and reagent pipetting; single use cuvettes are also employed. An analyte carry-over study was conducted to evaluate whether any significant amount of IFN-γ analyte is carried over from one sample into subsequent samples. The study used two types of samples:

a) Analyte Negative Sample - a heparinized plasma sample confirmed negative for IFN-γ, divided into five separate aliquots, and
b) Analyte Positive Sample - a heparinized plasma sample spiked with IFN-γ at a level of > 10 IU/mL.

Testing was performed using one LIAISON XL Analyzer, one lot of LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Study testing was conducted in two stages.
Stage A: Five aliquots of the negative sample were tested in duplicate in two separate runs to establish a mean signal of the negative sample.
Stage B: Analyte positive samples were tested in a series alternating with aliquots of analyte negative samples.

Study design for Stage A and Stage B are illustrated in Tables 14 and 15 below.

Table 14. Stage A Carry-Over Study Design

Stage A			
1st RUN		2nd RUN	
NEG Aliquot 1	2 replicates	NEG Aliquot 1	2 replicates
NEG Aliquot 1	2 replicates	NEG Aliquot 1	2 replicates
NEG Aliquot 2	2 replicates	NEG Aliquot 2	2 replicates
NEG Aliquot 2	2 replicates	NEG Aliquot 2	2 replicates
NEG Aliquot 3	2 replicates	NEG Aliquot 3	2 replicates
NEG Aliquot 3	2 replicates	NEG Aliquot 3	2 replicates
NEG Aliquot 4	2 replicates	NEG Aliquot 4	2 replicates
NEG Aliquot 4	2 replicates	NEG Aliquot 4	2 replicates
NEG Aliquot 5	2 replicates	NEG Aliquot 5	2 replicates
NEG Aliquot 5	2 replicates	NEG Aliquot 5	2 replicates

Table 15. Stage B Carry-Over Study Design

Stage B									
1st RUN		2nd RUN		3rd RUN		4th RUN		5th RUN	
HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate
NEG Aliquot 1	1 replicate	NEG Aliquot 1	1 replicate	NEG Aliquot 1	1 replicate	NEG Aliquot 1	1 replicate	NEG Aliquot 1	1 replicate
HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate
NEG Aliquot 2	1 replicate	NEG Aliquot 2	1 replicate	NEG Aliquot 2	1 replicate	NEG Aliquot 2	1 replicate	NEG Aliquot 2	1 replicate
HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate
NEG Aliquot 3	1 replicate	NEG Aliquot 3	1 replicate	NEG Aliquot 3	1 replicate	NEG Aliquot 3	1 replicate	NEG Aliquot 3	1 replicate
HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate
NEG Aliquot 4	1 replicate	NEG Aliquot 4	1 replicate	NEG Aliquot 4	1 replicate	NEG Aliquot 4	1 replicate	NEG Aliquot 4	1 replicate
HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate	HIGH POS	1 replicate
NEG Aliquot 5	1 replicate	NEG Aliquot 5	1 replicate	NEG Aliquot 5	1 replicate	NEG Aliquot 5	1 replicate	NEG Aliquot 5	1 replicate

The carry-over study summary results for Stage A and Stage B are illustrated below in Table 16 and Table 17, respectively.

Table 16. Stage A Carry-Over Study Summary Results

		Stage A			
		1st RUN		2nd RUN	
SAMPLE	REPLICATE	IU/mL	Result	IU/mL	Result
NEG Aliquot 1	Mean of Replicates 1 and 2	0.0239	neg	0.0307	neg
NEG Aliquot 1	Mean of Replicates 1 and 2	0.0365	neg	0.0361	neg
NEG Aliquot 2	Mean of Replicates 1 and 2	0.0241	neg	0.0217	neg
NEG Aliquot 2	Mean of Replicates 1 and 2	0.0232	neg	0.0320	neg
NEG Aliquot 3	Mean of Replicates 1 and 2	0.0276	neg	0.0333	neg
NEG Aliquot 3	Mean of Replicates 1 and 2	0.0251	neg	0.0358	neg
NEG Aliquot 4	Mean of Replicates 1 and 2	0.0205	neg	0.0306	neg
NEG Aliquot 4	Mean of Replicates 1 and 2	0.0364	neg	0.0302	neg
NEG Aliquot 5	Mean of Replicates 1 and 2	0.0325	neg	0.0250	neg
NEG Aliquot 5	Mean of Replicates 1 and 2	0.0248	neg	0.0270	neg
N° of samples tested		40			
N° of NEGATIVE results for all aliquots of negative sample		40 (100%)			
N° of POSITIVE results for all aliquots of negative sample		0			
% POSITIVE RESULTS		0%			

Table 17. Stage B Carry-Over Study Summary Results

SAMPLES	IU/mL	Result	IU/mL	Result	IU/mL	Result	IU/mL	Result	IU/mL	Result
HIGH POS Aliquot 1	10.0	POS	10.0	POS	10.0	POS	10.0	POS	10.0	POS
NEG Aliquot 1	0.03	neg	0.02	neg	0.03	neg	0.03	neg	0.03	neg
HIGH POS Aliquot 2	10.0	POS	10.0	POS	10.0	POS	10.0	POS	10.0	POS
NEG Aliquot 2	0.00	neg	0.03	neg	0.03	neg	0.02	neg	0.03	neg
HIGH POS Aliquot 3	10.0	POS	10.0	POS	10.0	POS	10.0	POS	10.0	POS
NEG Aliquot 3	0.02	neg	0.03	neg	0.03	neg	0.03	neg	0.02	neg
HIGH POS Aliquot 4	10.0	POS	10.0	POS	10.0	POS	10.0	POS	10.0	POS
NEG Aliquot 4	0.03	neg	0.03	neg	0.03	neg	0.03	neg	0.02	neg
HIGH POS Aliquot 5	10.0	POS	10.0	POS	10.0	POS	10.0	POS	10.0	POS
NEG Aliquot 5	0.03	neg	0.03	neg	0.03	neg	0.03	neg	0.03	neg

The percent negative results for all negative samples tested for the carry-over study was 100%, consistently demonstrating no significant amount of IFN-γ is carried over from positive samples into alternating negative samples in the series**.**

F. Sample Handling and Stability Studies

A series of studies were conducted to verify stability and consistency of IFN-γ detection of harvested plasma samples stored in secondary containers using a contrived sample test panel. Sample storage stability after multiple freeze-thaw cycles, as well as stability after storage at 20°C, -20°C, and 30°C ± 1°C was evaluated. Confirmed analyte negative lithium heparin plasma was collected from seven donors at DiaSorin (Italy). Plasma was divided into 7 aliquots; 6 aliquots were spiked with native IFN-γ, and one aliquot was not spiked to create test panel samples that spanned the assay measuring range as indicated below:

- 3 low-reactive samples (0.35 – 1 IU/mL)
- 3 reactive samples (1.0 – 10 IU/mL)
- 1 non-spiked sample (<0.35 IU/mL)

F.2 Freeze-Thaw Study

Each test panel sample was initially tested fresh to determine the T=0 value. Test panel samples were then divided into individual aliquots and frozen at -20°C. Aliquots were subjected to one, two, three, four, and five freeze-thaw cycles. Sample aliquots were thawed at room temperature; once completely thawed were refrozen. Testing of all sample aliquots was then performed on a single occasion using one lot of LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Study results were evaluated as follows:

- Calculation of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each of the subsequent freeze-thaw cycles, for each sample.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent freeze-thaw cycle number for low-reactive and reactive samples.

- Fitting a regression line to the data to determine the freeze-thaw cycle at which the regression line exceeds ± 10% difference from T=0.
- Calculation of %CV across freeze-thaw cycles.

The stability of low-reactive and reactive samples was determined to be the freeze-thaw cycle that was one cycle less than the last freeze-thaw cycle tested before the regression line exceeds ±10% difference from T=0. The stability of non-reactive samples was considered acceptable if the non-reactive status of the sample is maintained one freeze-thaw cycle beyond the claimed freeze-thaw cycle.

Based on the real-time study results, plasma sample **freeze-thaw stability is determined to be four freeze-thaw cycles** for both reactive and non-reactive samples. This finding is reflected in the LIAISON QuantiFERON-TB Gold Plus assay package insert.

F.3 Sample Storage Study 2-8°C

Each test panel sample was initially tested fresh to determine the T=0 value. Test panel samples were then divided into individual sample aliquots and stored at 2-8°C for 7, 14, 21, 22, 28, 29, 30, and 44 days. All sample aliquots that were stored at different exposure times were then each tested in duplicate using one lot of LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Study results were evaluated as follows:
- Calculation of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point, for each sample.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point for low-reactive and reactive samples.
- Fitting a regression line to the data to determine the storage time point at which the regression line exceeds ± 10% difference from T=0.
- Calculation of %CV across the storage time points.

The stability of low-reactive and reactive samples was determined to be the storage time point that was one cycle less than the last storage time point tested before the regression line exceeds ±10% difference from T=0. The stability of non-reactive samples was considered acceptable if the non-reactive status of the sample is maintained one storage time point beyond the claimed storage time point.

Based on the real-time study results, plasma sample **stability when stored at 2-8°C is determined to be 28** days for both reactive and non-reactive samples. This finding reflected in the LIAISON QuantiFERON-TB Gold Plus assay package insert.

F.4 Sample Storage Study -20°C

Each test panel sample was initially tested fresh to determine the T=0 value. Test panel samples were then divided into individual sample aliquots and stored at -20°C for 1, 3, 4, 6, and 7 months. All sample aliquots that were stored at different exposure times were then each tested in duplicate using one lot of LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Study results were evaluated as follows:

- Calculation of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point, for each sample.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point for low-reactive and reactive samples.
- Fitting a regression line to the data to determine the storage time point at which the regression line exceeds ± 10% difference from T=0.
- Calculation of %CV across the storage time points.

The stability of low-reactive and reactive samples was determined to be the storage time point that was one cycle less than the last storage time point tested before the regression line exceeds ±10% difference from T=0. The stability of non-reactive samples was considered acceptable if the non-reactive status of the sample is maintained one storage time point beyond the claimed storage time point.

Based on the real- time study results, plasma **sample stability when stored at -20°C is determined to be 6 months** for both reactive and non-reactive samples. This finding is reflected in the LIAISON QuantiFERON-TB Gold Plus assay package insert.

F.5 Sample Storage Study 30°C ± 1°C

Each test panel sample was initially tested fresh to determine the T=0 value. Test panel samples were then divided into individual sample aliquots and stored at 30°C ± 1°C for 8, 9, 24, 25 hours. All sample aliquots that were stored at different exposure times were then each tested in duplicate using one lot of LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Study results were evaluated as follows:

- Calculation of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point, for each sample.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the fresh condition versus the amount of detected IFN-γ at each subsequent storage time point for low-reactive and reactive samples.
- Fitting a regression line to the data to determine the storage time point at which the regression line exceeds ± 10% difference from T=0.

- Calculation of %CV across the storage time points.

The stability of low-reactive and reactive samples was determined to be the storage time point that was one cycle less than the last storage time point tested before the regression line exceeds ±10% difference from T=0. The stability of non-reactive samples was considered acceptable if the non-reactive status of the sample is maintained one storage time point beyond the claimed storage time point.

Based on the real- time study results, plasma **sample stability when stored at** 30°C ± 1°C **is determined to be 24 hours** for both reactive and non-reactive samples.

G. <u>LIAISON QuantiFERON-TB Gold Plus Assay Calibration Stability Studies</u>

A study was conducted to assess the stability of the LIAISON QuantiFERON-TB Gold Plus assay calibration by simulating normal conditions of use. A coded test panel of six samples was prepared by spiking analyte negative lithium heparinized plasma samples with native IFN-γ, to achieve concentrations that spanned the assay result range. Native IFN-γ was obtained from plasma harvested after incubation/stimulation of whole blood in QFT-Plus Mitogen blood collection tubes.

LIAISON QuantiFERON-TB Gold Plus assay reagent integrals were opened and stored on board the LIAISON XL Analyzer in the refrigerated reagent bay.

Testing of samples was performed in duplicate, using one lot of the LIAISON QuantiFERON-TB Gold Plus assay kit, and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

The performance of the LIAISON QuantiFERON-TB Gold Plus assay kit, using the open reagent integrals was evaluated weekly for a period of nine weeks. Sample and control test results were generated using the initial (Time 0) assay calibration, and using a freshly reconstituted conjugate vial every 14 days. A second calibration stability study, using the same study design, but using a freshly reconstituted conjugate for each run was also conducted.

Study results were evaluated as follows:
- The IU/mL value result, for each sample, was evaluated and compared to the defined acceptance criteria. The acceptance criteria were the sole means of evaluation for non-reactive samples.
- Calculation of the mean % difference in the amount of detected IFN-γ (in IU/mL) of each sample at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of each reactive sample at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Fitting a regression line to the sample data to determine the time point at which the regression line exceeds ± 10% difference from T=0.

The calibration stability was determined to be the time point that was one point less than the final time point tested before the regression line exceeds ± 10% difference from T=0 for each reactive sample.

Regarding the initial calibration interval study that used a freshly reconstituted conjugate vial every 14 days, for the majority of samples tested, the fitted regression line did not exceed ± 10% deviation from T=0 over 9 weeks of testing. However, for one sample the fitted regression line intercepted the -10% deviation from T=0 between the Week 8 and Week 9 time points.

Regarding the second calibration interval study that used a freshly reconstituted conjugate for each run, for all samples tested, the fitted regression line did not exceed ± 10% deviation from T=0 over the entire 9 weeks of testing.

Based on the findings of both calibration interval studies, the stability of the LIAISON QuantiFERON-TB Gold Plus assay was determined to be 7 weeks. However, in an effort to foster a degree of consistency across assay kit reagents for ease of customer use, the **stability of the LIAISON QuantiFERON-TB Gold Plus assay calibration was established as 4 weeks.**

H. LIAISON QuantiFERON-TB Gold Plus Reagent and LIAISON Control QuantiFERON-TB Gold Plus Storage and Shelf Life Studies

H.1. LIAISON QuantiFERON-TB Gold Plus Reagent Storage and Shelf Life Study

A (real-time) study was conducted to evaluate the long term stability of the LIAISON QuantiFERON-TB Gold Plus assay kit reagents and to establish shelf life. Testing was conducted using a coded test panel of samples and controls (level 1 and level 2). The samples were prepared by spiking analyte negative lithium heparinized plasma samples with native IFN-γ, to achieve concentrations that spanned the assay result range. Native IFN-γ was obtained from plasma harvested after incubation/stimulation of whole blood in QFT-Plus Mitogen blood collection tubes.

Testing was performed in duplicate using three lots of LIAISON QuantiFERON-TB Gold Plus assay kits, and three lots of LIAISON Control QuantiFERON-TB Gold Plus kits.

The performance of the LIAISON QuantiFERON-TB Gold Plus assay kit was evaluated at time points of 1, 2, 4, 6, 9, 11, 12, 13, and 14 months after kit manufacture.

Data analysis of the study test panel sample and control results included the following:

For test panel samples:

- Data for each sample tested, on each kit lot, was plotted separately.
- The mean amount of detected IFN-γ was plotted against the corresponding study time point for each sample.

- A linear regression was fitted to the test sample data.
- The y-intercept of the regression line was calculated to estimate the baseline value for the IFN-γ.
- The 95% confidence intervals for each time point were calculated.
- ± 10% deviation from the regression intercepts were indicated on the plots as horizontal lines.

Similarly for controls:

- The mean amount of detected IFN-γ (IU/mL) for each of the controls, at each testing point, was evaluated by comparing it to the established range, as defined in the product Certificate of Analysis (CoA).
- Data for each control tested, on each kit lot, was plotted separately.
- The mean amount of detected IFN-γ was plotted against the corresponding study time point for each control.
- A linear regression was fitted to the test sample data.
- The y-intercept of the regression line was calculated to estimate the baseline value for the IFN-γ.
- ± 10% deviation from the regression intercepts were indicated on the plots as horizontal lines.

The LIAISON QuantiFERON-TB Gold Plus assay kit reagent shelf life claim was determined to be the time point that is one time point less than the final time point tested before the regression line exceeds ± 10% difference from the baseline value.

The test results of all three lots of positive and negative controls fell within the established range as defined in the product CoA. Regarding all three lots of positive controls tested, the fitted regression line did not exceed ± 10% deviation from the regression intercept at any time point during the study. Regarding all three lots of negative controls tested, due to the very low result values, the absolute differences from the regression intercept, at each time point was evaluated, and found to minimal and acceptable.

Based on the real-time stability study findings, the claimed **shelf life of the LIAISON QuantiFERON-TB Gold Plus assay was established at 13 months after the date of manufacture**.

H.2. LIAISON Control QuantiFERON-TB Gold Plus Kit Storage and Shelf Life Study

A (real-time) study was conducted to evaluate the long term stability of the LIAISON Control QuantiFERON-TB Gold Plus kit and to establish shelf life. Testing was performed in duplicate using three lots of LIAISON QuantiFERON-TB Gold Plus assay kits, and three lots of LIAISON Control QuantiFERON-TB Gold Plus kits.

The performance of the LIAISON Control QuantiFERON-TB Gold Plus kit was evaluated at time points of 1, 2, 4, 6, 9, 11, 12, 13, and 14.5 months after kit manufacture.

Data analysis of the study control results included the following:

- The mean amount of detected IFN-γ (IU/mL) for each of the controls, at each testing point, was evaluated by comparing it to the established range, as defined in the product Certificate of Analysis (CoA).
- Data for each control tested, on each kit lot, was plotted separately.
- The mean amount of detected IFN-γ (IU/mL) was plotted against the corresponding study time point for each control.
- A linear regression was fitted to the control data.
- The y-intercept of the regression line was calculated to estimate the baseline value for the IFN-γ.
- The 95% confidence intervals for each time point were calculated.
- ± 10% deviation from the regression intercepts were indicated on the plots as horizontal lines.

The LIAISON Control QuantiFERON-TB Gold Plus kit shelf life claim was determined to be the time point that is one time point less than the final time point tested before the regression line exceeds ± 10% difference from the baseline value, and also within the established range, as defined in the CoA.

The test results of all three lots of positive and negative controls fell within the established range as defined in the product CoA. Regarding all three lots of positive controls tested, the fitted regression line did not exceed ± 10% deviation from the regression intercept at any time point during the study. Regarding all three lots of negative controls tested, due to the very low result values, the absolute differences from the regression intercept, at each time point was evaluated, and found to be minimal and acceptable.

Based on the real-time stability study findings, the claimed **shelf life of the LIAISON Control QuantiFERON-TB Gold Plus kit was established at 13 months after the date of manufacture when stored at 2-8°C**.

I. Open Reagent Kit Stability Studies

Studies were conducted to evaluate the stability of the LIAISON QuantiFERON-TB Gold Plus kit reagents by simulating normal conditions of use. The simulation included refrigerated storage of the reagent integral (Magnetic Particles, Diluent, and Assay Buffer W) on board LIAISON XL Analyzer for the duration of the study, and reconstitution and refrigerated storage of lyophilized kit reagents (Calibrator A, Calibrator B, Buffer R, and Conjugate) to mimic a customer's routine. Conjugate reconstituted at T=0 was kept for at least six hours on board the analyzer, then stored at 2-8°C. Controls and calibrators reconstituted at T=0 were kept at least four hours on board the analyzer, then stored at 2-8°C.

Testing was conducted using a coded test panel of samples and controls (level 1 and level 2). The samples were prepared by spiking analyte negative lithium heparinized plasma samples with native IFN-γ, to achieve concentrations that spanned the assay result range. Native IFN-γ was obtained from plasma harvested after incubation/stimulation of whole blood in QFT-Plus Mitogen blood collection tubes. Samples were tested in duplicate

using one lot of LIAISON QuantiFERON-TB Gold Plus assay kit and one lot of LIAISON Control QuantiFERON-TB Gold Plus.

Data analysis of the study results included the following:

- The IU/mL value result, for each reactive sample and control was evaluated and compared to the defined acceptance criteria. The acceptance criteria were the sole means of evaluation for non-reactive samples and controls.
- Calculation of the mean % difference in the amount of detected IFN-γ (in IU/mL) of each reactive sample and control at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of each reactive sample and control at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Fitting a regression line to the sample data to determine the time point at which the regression line exceeds ± 10% difference from T=0.

Open reagent stability was determined to be the time point that was one time point less than the final time point tested before the regression line exceeded ± 10% difference from T=0 for each reactive sample.

I.1. Open Conjugate Stability

The performance of the conjugate was evaluated at the following time points after reconstitution: Day 0, 3, 7, 10, 14, 18, 21, and 32. For all reactive samples and controls tested, the fitted regression line did not exceed ± 10% deviation from T=0 over 32 days of testing. The non-reactive samples and controls met the defined acceptance criteria from T=0 over 32 days of testing.

Based on the study findings, the open stability of the conjugate was determined to be 21 days. However, in an effort to foster a degree of consistency across assay kit reagents for ease of customer use, the **stability of the open conjugate was established at 14 days when stored at 2-8°C**.

I.2. On board Reagent Integral Stability Study

The performance of the reagent integral was evaluated at the following at time points after opening: Week 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9. However, due to the limitation of the calibration stability of 7 weeks, the analysis of study data was limited to 7 weeks. For all reactive samples and controls tested, the fitted regression line did not exceed ± 10% deviation from T=0 over 7 weeks of testing. The non-reactive samples and controls met the defined acceptance criteria from T=0 over 7 weeks of testing.

Based on the study findings, the open stability of the reagent integral was determined to be 6 weeks. However, in an effort to foster a degree of consistency across assay kit reagents for ease of customer use, the **stability of the open reagent integral was established at 4 weeks when stored at 2-8°C**.

I.3. Open Calibrators Stability Study

The performance of the calibrators was evaluated at the following time points after opening: Week 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9. However, due to the limitation of the on-board reagent integral stability of 5 weeks, the analysis of study data was limited to 5 weeks. For the majority of reactive samples and controls tested, the fitted regression line did not exceed ± 10% deviation from T=0 over 5 weeks of testing. The non-reactive samples and controls met the defined acceptance criteria from T=0 over 5 weeks of testing.

Based on the study findings, the open stability of the calibrators was determined to be 5 weeks. However, in an effort to foster a degree of consistency across assay kit reagents for ease of customer use, the **stability of the open calibrators was established at 4 weeks when stored at 2-8°C**.

J. Open Control Kit Stability Studies

Studies were conducted to evaluate the stability of the LIAISON Control QuantiFERON-TB Gold Plus kit by simulating normal conditions of use. Controls were opened and reconstituted at T=0, stored on the LIAISON XL Analyzer for at least four hours, then stored at 2-8°C. Controls were tested in duplicate, weekly for a total of nine weeks. However due to the limitation of the on-board reagent integral stability of 5 weeks, the analysis of study data was limited to 5 weeks.

Data analysis of the study results included the following:

- The IU/mL value result, for each control was evaluated and compared to the defined acceptance criteria. The acceptance criteria were the sole means of evaluation for the non-reactive control.
- Calculation of the mean % difference in the amount of detected IFN-γ (in IU/mL) of the reactive control at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Plotting of the mean % difference between the amount of detected IFN-γ (in IU/mL) of the control at Time 0 versus the amount of detected IFN-γ at each subsequent time point.
- Fitting a regression line to the control data to determine the time point at which the regression line exceeds ± 10% difference from T=0.

Open control stability was determined to be the time point that was one time point less than the final time point tested before the regression line exceeded ± 10% difference from T=0 for each reactive control. The fitted regression line did not exceed ± 10% deviation from T=0 over 5 weeks of testing for the reactive control. The non-reactive control met the defined acceptance criteria from T=0 over 5 weeks of testing.

Based on the study findings, the open stability of the controls was determined to be 5 weeks. However, in an effort to foster a degree of consistency across assay kit reagents for ease of customer use, the **stability of the open controls was established at 4 weeks when stored at 2-8°C**.

K. Reagent and Control Transport Stability Studies

Studies were conducted to verify that LIAISON QuantiFERON-TB Gold Plus assay reagents, and LIAISON Control QuantiFERON-TB Gold Plus maintain performance after transport and delivery to customers.

K.1. LIAISON QuantiFERON-TB Gold Plus Assay Reagent Kit Transport Stability Study

The shipment of the LIAISON QuantiFERON-TB Gold Plus assay reagent kit includes two parts:

Part One: Intercontinental shipment from DiaSorin S.p.A in Saluggia, Italy to DiaSorin Inc., in Stillwater, Minnesota. The product is shipped under storage conditions from 0-30°C and may take 2 to 10 days depending on duration of customs clearance. During the shipment, temperature exposure is monitored with a temperature data logger. In the event that the product is found to have been exposed to temperatures outside the range of 0-30°C, the product is deemed non-conformant and an investigation is initiated.

Part Two: Shipment within the United States from DiaSorin Inc., in Stillwater, Minnesota to the end user. This leg of the shipment is at ambient temperature (up to 35.6°C) and occurs overnight (up to a total of 27 hours) or worst case, over a few days.

In this study, reagent transport was simulated according to the following conditions:

- All assay kit reagents[4] stored for five days (at least 120 hours) at 30°C, representing potential of removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to room temperature (18-24°C) conditions for up to 5 days;
- All assay kit reagents stored for ten days (at least 240 hours) at 30°C, representing potential removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to room temperature (18-24°C) conditions for up to 10 days;
- All assay kit reagents stored for one day (at least 24 hours) at 37°C, representing potential removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to extreme temperature conditions for a short period of time, up to 1 day;
- All assay kit reagents stored for one day (at least 24 hours) at -20°C, representing the possible freezing of product.

The sample test panel used during the study was comprised of three samples:

- High-Negative (0.20 – 0.29 IU/mL)
- Close to Medical Decision Point (0.30 – 0.35 IU/mL)
- Positive (4.0 – 6.0 IU/mL)

These samples were prepared by spiking confirmed analyte-negative human (heparinized) plasma samples with native IFN-γ. Native IFN-γ was obtained via incubation of whole

[4]All assay kit reagents includes: Reagent Integral, Conjugate, Calibrator A, Calibrator B, and Buffer R

blood in QFT-Plus Mitogen blood collection tubes, and subsequently stimulated plasma with analyte IFN-γ was harvested.

The study was performed using one lot of the LIAISON QuantiFERON-TB Gold Plus assay kit and one lot of the LIAISON Control QuantiFERON-TB Gold Plus. Testing was performed once during the life of the assay kit (i.e., at the one month time point). Each simulated stress condition was performed versus the control condition (i.e., product stored at 2-8°C). For each of the four stress conditions, and the control condition, a test result was determined by taking the mean of 12 sample test results generated over four days, with one run per day, and three replicates per run. The study design summary is illustrated in Table 18 below.

Table 18. LIAISON QuantiFERON-TB Gold Plus Assay Reagent Kit Transport Stability Study Design Summary

Sample	Day 1 – 1 Run	Day 2 – 1 Run	Day 3– 1 Run	Day 4– 1 Run	Data point for testing occasion
High Negative	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Mean of 12 replicates
	Replicate 2	Replicate 2	Replicate 2	Replicate 2	
	Replicate 3	Replicate 3	Replicate 3	Replicate 3	
Close to the Medical Decision Point	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Mean of 12 replicates
	Replicate 2	Replicate 2	Replicate 2	Replicate 2	
	Replicate 3	Replicate 3	Replicate 3	Replicate 3	
Positive	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Mean of 12 replicates
	Replicate 2	Replicate 2	Replicate 2	Replicate 2	
	Replicate 3	Replicate 3	Replicate 3	Replicate 3	

The results of each sample tested for each stressed condition were evaluated in terms of the percent deviation from the unstressed reference condition. Results were considered acceptable if the amount of detected IFN-γ for each stress condition did not exceed ±10% of the amount of detected IFN-γ for the unstressed condition. A summary of the LIAISON QuantiFERON-TB Gold Plus assay reagent kit transport stability study results is illustrated in Table 19 below.

Table 19. LIAISON QuantiFERON-TB Gold Plus Assay Reagent Kit Transport Stability Study Summary Results

Sample	Stress condition:	REFERENCE (2-8 °C)	5 DAYS @ 30 °C	10 DAYS @ 30 °C	1 DAY @ 37 °C	1 DAY @ -20 °C
High Negative	MEAN IU/mL	0.235	0.237	0.236	0.225	0.242
	Percent Deviation from Unstressed Condition		0.67%	0.14%	-4.18%	2.80%
Close to Medical Decision Point	MEAN IU/mL	0.299	0.319	0.319	0.294	0.319
	Percent Deviation from Unstressed Condition		6.92%	6.75%	-1.54%	6.89%
Positive	MEAN IU/mL	4.02	4.08	4.37	4.28	4.10
	Percent Deviation from Unstressed Condition		1.35%	8.57%	6.25%	1.84%

The acceptance criterion was met for all stress conditions tested demonstrating that the LIAISON Control QuantiFERON-TB Gold Plus assay reagents maintain acceptable performance after simulations that mimic product transport conditions.

K.2. LIAISON Control QuantiFERON-TB Gold Plus Kit Transport Stability Study

The shipment of the LIAISON Control QuantiFERON-TB Gold Plus kit includes two parts:

Part One: Intercontinental shipment from DiaSorin S.p.A in Saluggia, Italy to DiaSorin Inc., in Stillwater, Minnesota. The product is shipped under storage conditions from 0-30°C and may take from 2 to 10 days depending on duration of customs clearance. During the shipment, temperature exposure is monitored with a temperature data logger. In the event that the product is found to have been exposed to temperatures outside the range of 0-30°C, the product is deemed non-conformant and an investigation is initiated.

Part Two: Shipment within the United States from DiaSorin Inc., in Stillwater, Minnesota to the end user. This leg of the shipment is at ambient temperature (up to 35.6°C) and is expected to occur overnight (up to a total of 27 hours) or worst case, over a few days.

In this study, reagent transport was simulated according to the following conditions:

- Controls stored for five days (at least 120 hours) at 30°C, representing potential of removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to room temperature (18-24°C) conditions for up to 5 days;
- Controls stored for ten days (at least 240 hours) at 30°C, representing potential removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to room temperature (18-24°C) conditions for up to 10 days;
- Controls stored for one day (at least 24 hours) at 37°C, representing potential removal from controlled refrigerated storage conditions (2 – 8°C) and exposure to extreme temperature conditions for a short period of time, up to 1 day;
- Controls stored for one day (at least 24 hours) at -20°C, representing the possible freezing of product.

The study was performed using one lot of the LIAISON QuantiFERON-TB Gold Plus assay kit and one lot of the LIAISON Control QuantiFERON-TB Gold Plus. Testing was performed once during the life of the control kit (i.e., at the one month time point). Each simulated stress condition was performed versus the control condition (i.e., product stored at 2-8°C). For each of the four stress conditions, and the control condition, a test result was determined by taking the mean of 12 test results generated over four days, with one run per day, and three replicates per run. The study design summary is illustrated in Table 20 below.

Table 20. LIAISON Control QuantiFERON-TB Gold Plus Kit Transport Stability Study Design Summary

Control	Day 1 – 1 Run	Day 2 – 1 Run	Day 3– 1 Run	Day 4– 1 Run	Data point for testing occasion
Negative Control RS 912	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Mean of 12 replicates
	Replicate 2	Replicate 2	Replicate 2	Replicate 2	
	Replicate 3	Replicate 3	Replicate 3	Replicate 3	
Positive Control RS 914	Replicate 1	Replicate 1	Replicate 1	Replicate 1	Mean of 12 replicates
	Replicate 2	Replicate 2	Replicate 2	Replicate 2	
	Replicate 3	Replicate 3	Replicate 3	Replicate 3	

The results of the controls (Negative RS 912, Positive RS 914) tested for each stress condition were evaluated in terms of the percent deviation from the unstressed reference condition. Results were considered acceptable if the amount of detected IFN-γ of each stressed condition did not exceed ±10% of the amount of detected IFN-γ of the unstressed condition. A summary of the LIAISON Control QuantiFERON-TB Gold Plus kit transport stability study results is illustrated in Table 21 below.

Table 21. LIAISON Control QuantiFERON-TB Gold Plus Kit Transport Stability Study Summary Results

Control	Stress condition	REFERENCE (2-8 °C)	5 DAYS @ 30 °C	10 DAYS @ 30 °C	1 DAY @ 37 °C	1 DAY @ -20 °C
RS 912 Negative Control	MEAN IU/mL	0.042	0.041	0.038	0.041	0.043
	Percent Deviation from Unstressed Condition		-1.87%	-7.87%	-1.40%	3.65%
RS 914 Positive Control	MEAN IU/mL	0.96	1.01	0.96	1.00	1.00
	Percent Deviation from Unstressed Condition		4.78%	-0.31%	4.01%	4.62%

The acceptance criterion for all stress conditions tested was met demonstrating that the LIAISON Control QuantiFERON-TB Gold Plus kit maintains acceptable performance after simulations that mimic product transport conditions..

L. Software

A software review was conducted, and all documentation provided was found to be appropriate and acceptable. A summary of the elements of the review are as follows:

- Level of Concern: Moderate; the level of concern is acceptable
- Device Hazard Analysis: Relevant software hazards were identified, and detailed risk assessments and mitigations were provided.
- Verification and Validation: Verification and validation plans, logs, and result summaries were provided for all versions of instrument software.
- Revision Level History: This is the first version of the LIAISON QuantiFERON Software to be submitted for review.
- Unresolved Anomalies: Documentation of all unresolved anomalies was found to be acceptable. Residual issues do not impact assay safety or effectiveness.

A cybersecurity review was also conducted and found to be compliant/acceptable.

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Clinical Study Design

A multi-site clinical agreement study was conducted comparing the LIAISON QuantiFERON-TB Gold Plus assay to the QIAGEN QuantiFERON-TB Gold Plus (QFT-Plus). Samples were collected and tested from study subjects who had signs and symptoms consistent with active TB disease (Active TB cohort), subjects who had no identified risk factors for tuberculosis infection (Low Risk cohort), and subjects with at least one known risk factor for tuberculosis exposure and at risk for latent TB infection (Mixed Risk cohort).

A.1 Clinical Performance Study Analysis

There is no definitive reference method for confirming or excluding the diagnosis of latent tuberculosis infection (LTBI); accordingly an estimate of sensitivity and specificity of the LIAISON QuantiFERON-TB Gold Plus assay cannot be directly evaluated. Therefore, the specificity of the LIAISON QuantiFERON-TB Gold Plus assay was approximated by testing plasma samples from a cohort of study subjects with no known risk factors of tuberculosis infection (low risk). Similarly, sensitivity was approximated by testing plasma samples from a cohort of study subjects with culture confirmed active tuberculosis (TB) disease. Assay performance was further evaluated by testing plasma samples from a cohort of healthy study subjects with identified risk factors for LTBI (mixed risk). Comparative performance of the LIAISON QuantiFERON-TB Gold Plus relative to QIAGEN QuantiFERON-TB Gold Plus test was also evaluated.

A.2 Clinical Inclusion and Exclusion Criteria

Inclusion Criteria

Active TB Cohort

- Subjects with clinical symptoms consistent with suspicion for active TB disease;
- Subjects receiving, or are likely to receive, therapy for active TB (therapy must not have been initiated more than 2 weeks before recruitment into the study);
- Sputum and/or other relevant bodily samples collected from these individuals for AFB smear and culture testing to determine the presence of *M. tuberculosis* organisms. Alternatively, FDA approved nucleic acid amplification (NAA) methods in combination with culture testing were used to confirm the presence of *M. tuberculosis* organisms.

Low Risk Cohort

- Individuals with no identified risk factors for tuberculosis infection.

Mixed Risk Cohort

- Subjects with at least one of the following known risk factors for latent TB infection (LTBI)
  - Recent contact with a known source case.
  - Inmate at a correctional facility.
  - Resident of a homeless shelter or nursing home.
  - Healthcare worker.
  - Time spent in a country with a high prevalence of tuberculosis.
- Subjects deemed free from tuberculosis disease and exhibiting no clinical signs/symptoms consistent with active tuberculosis disease.
- In addition, this group also included subjects who are HIV positive, and subjects who have been BCG vaccinated.

Exclusion Criteria

Active TB Cohort

- Subjects who have taken therapy for active tuberculosis infection or latent TB infection for more than 14 days.
- Subjects for which culture confirmation of *M. tuberculosis* was not obtained.
- Subjects aged less than 18 years or greater than 80 years.

Low Risk Cohort

- Subjects who have resided for more than one (1) month in an area with a current active TB rate of >50/100,000
- Subjects who have lived, volunteered, or worked for longer than one (1) month in a nursing home, hospital, homeless shelter, or other situation known to be associated with an increased risk of TB exposure.
- Individuals having exposure to someone who was sick with TB.
- Subjects with a previous history of TB diagnosis or treatment for TB.
- Subjects having immunosuppressive conditions that could interfere with a person's ability to initiate a cell mediated immune response in the assay such as the following:
  - HIV infection
  - Cancer requiring chemotherapy in the preceding 3 months
  - Rheumatoid arthritis
  - Renal failure
  - Systemic immunosuppressive usage
- Subjects aged less than 18 years or greater than 80 years.

Mixed Exposure Risk Cohort

- Subjects not having at least one of the known risk factors for tuberculosis exposure (see Inclusion Criteria, Mixed Risk).
- Subjects presenting with clinical signs and symptoms consistent with active tuberculosis disease.
- Subjects aged less than 18 years or greater than 80 years.

Eligibility for each subject group was assessed by using Case Report Forms (CRFs).

A.3 Follow-Up Schedule
Not Applicable

A.4 Clinical Endpoints

With regard to safety, the results of the clinical agreement study show the LIAISON QuantiFERON - TB Gold Plus assay and the LIAISON Control QuantiFERON - TB Gold Plus pose no safety hazards to the patient as results from this *in vitro* diagnostic device are used as an aid in the diagnosis of *M. tuberculosis* infection.

With regard to effectiveness the results of the clinical agreement study show the LIAISON QuantiFERON - TB Gold Plus assay and the LIAISON® Control QuantiFERON - TB Gold Plus are effective in providing a result that is sufficient to aid in the diagnosis of TB infection.

B. Accountability of PMA Cohort

Active TB Cohort – Accountability
Samples from 189 study subjects from four (4) different countries (Mexico, Italy, Uganda, and Ukraine) were collected. A total of 29 samples were excluded from analysis; (19) were TB culture negative, (2) were acid-fast bacilli smear/PCR negative, (2) due to unavailability of TB culture result, (1) due to TB culture contamination, (1) due to lack of TB culture confirmation, and (4) due to invalid test results on the LIAISON XL Analyzer. A total of 160 samples were ultimately included in the clinical performance analysis, including 109 prospectively collected samples, and 51 retrospectively collected samples.

Low Risk Cohort - Accountability
Samples from 389 study subjects from the United States (West, Midwest, and Southeast) were prospectively collected. A total of 77 samples were excluded from analysis due to indeterminate results attributed to a problem with sample collection/processing. A total of 312 samples were ultimately included in the clinical performance analysis.

Mixed Risk Cohort -Accountability
Samples from 688 study subjects from the United States (West, Northeast) were prospectively collected. A total of 82 samples were collected but not tested because a sufficient number of samples for analysis were already obtained prior to receipt of these samples at DiaSorin. In addition, 36 samples were excluded from analysis; (17) due to compromise of shipment, (12) due to processing technical error, (2) due to labeling errors, (2) due to insufficient sample quantity, (1) due to improper consent, (1) due to missing sample tube, and (1) due to TB2 Failure XL Analyzer error. A total of 570 samples were ultimately included in the clinical performance analysis.

C. Clinical Study Population Demographics

The demographics of the study population illustrated in Tables A and B below are representative of' a clinical study performed in the United States.

Table A. Summary of Clinical Study Demographics (Race, Gender)

	Active TB		Low Risk		Mixed Risk	
Race	**N**	**%**	**N**	**%**	**N**	**%**
American Indian/ Alaskan Native	0	0.0%	0	0.0%	6	1.1%
Asian	7	4.4%	0	0.0%	6	1.1%
Black/African American	45	28.1%	299	95.8%	321	56.3%
Native Hawaiian or Other Pacific Islander	0	0.0%	0	0.0%	0	0.0%
White	85	53.1%	13	4.2%	225	39.5%
Unknown	14	8.8%	0	0.0%	5	0.9%
Other	9	5.6%	0	0.0%	7	1.2%
Total	160	100.0%	312	100.0%	570	100.0%
Gender	**N**	**%**	**N**	**%**	**N**	**%**
Female	45	28.1%	57	18.3%	188	33.0%
Male	115	71.9%	255	81.7%	382	67.0%
Total	160	100.0%	312	100.0%	570	100.0%

Table B. Summary of Clinical Study Demographics (Age)

Cohort	Active TB			Low Risk			Mixed Risk		
Gender	**N**	**Age Range (yrs)**	**Average (yrs)**	**N**	**Age Range (yrs)**	**Average (yrs)**	**N**	**Age Range (yrs)**	**Average (yrs)**
Female	45	19-85*	40	57	18-62	36.8	188	19-72	44.3
Male	115	18-85*	41	255	18-65	38	382	20-79	46.8
Total	160			312			570		

Of the entire 1042 clinical study subject population, the percentage of each race was as follows: American Indian/Alaskan Native (0.6%), Asian (1.3%), Black/African American (63.8%), Caucasian (31.0%), Other (1.5%), and Unknown (1.8%) ethnicities.

A summary of the demographics of each clinical study cohort are as follows:

- Individuals with signs and symptoms of TB infection (Active TB cohort): [160 total – 71.9% male (n=115), ages 18-85 years, 2 unknown age; 28.1% female (n=45), ages 19-85 years, 2 unknown age]
- Individuals with no identified risk factors (Low Risk cohort) for TB infection: [312 total – 81.7% male (n=255), ages 18-65 years; 18.3% female (n=57), ages 18-62 years]

- Individuals at risk for TB infection due to medical conditions, occupation, known exposure event, and individuals with a higher probability of TB infection due to living in areas endemic for TB (Mixed Risk cohort):
  [570 total – 67% male (n=382), ages 20-79 years; 33% female (n=188), ages 19-72 years]

Clinical Sample Collection and Handling

Whole blood samples were collected by several approved vendors (Table 22) across multiple collections sites, in accordance with the QFT-Plus assay package insert, using one of two methods of blood collection, i.e., direct draw into QFT-Plus blood collection tubes, or collection into lithium heparin tube followed by transfer of 1mL aliquots to each of the QFT-Plus blood collection tubes. Aliquots of plasma harvested from each of the QFT-Plus blood collection tubes were stored at -20°C or lower and sent to DiaSorin Inc. on dry-ice in batch shipments. Samples were de-identified/delinked from the study subject and randomly distributed across the clinical testing sites (Table 23).

Table 22. Commercial Vendor/Sample Collection Sites

Cohort	Vendor Name (Vendor Location)	Collection Location	Collection Dates
Active TB	iSpecimen (Lexington, MA)	Columbia, Ukraine	None Received
Active TB	Discovery Life Sciences (Los Osos, CA)	Mexico, Uganda	May 2018 through September 2018
Active TB	Cerba Xpert (France)	Ukraine	May 2018 through August 2018
Active TB	AVR (Italy)	Italy	April 2018 through August 2018
Active TB	San Raffaele Hospital (Italy)	Italy	May 2018 through September 2018
Active TB	SLR Research Corporation (Carlsbad, CA)	Mexico	August 2018
Low Risk	Boca Biolistics (Pompano Beach, FL)	USA Southeast	June 2018 through July 2018
Low Risk	Discovery Life Sciences (Los Osos, CA)	USA Midwest	May 2018 through October 2018
Mixed Risk	Boca Biolistics (Pompano Beach, FL)	USA West	June 2018 through August 2018
Mixed Risk	Discovery Life Sciences (Los Osos, CA)	USA Northeast	May 2018 through August 2018
Mixed Risk	iSpecimen (Lexington, MA)	USA West and Northeast	April 2018 through August 2018

In the clinical study, a total of 1042 plasma samples were tested and included in the clinical study performance analysis, to including 160 samples in the *Active TB* cohort, 312 samples in the *Low Risk* cohort, and 570 samples in the *Mixed Risk* cohort. The LIAISON QuantiFERON-TB Gold Plus assay testing was conducted at two U.S. external testing sites (i.e., ARDL, KMI), and internally at both DiaSorin Inc. (U.S.) and DiaSorin S.p.A (Italy). All testing was conducted using the LIAISON XL Analyzer with software version 4.2.0.2. Test results were calculated manually as positive, negative, or indeterminate at the DiaSorin S.p.A. study test site. Test results were calculated as positive, negative, or indeterminate using the LIAISON QuantiFERON Software (LQS) at ARDL, KMI, and DiaSorin Inc. The LIAISON Control QuantiFERON-TB Gold Plus (Level 1 and Level 2) was run on each day of testing at all clinical testing sites. All control testing during the course of the clinical study met acceptance criteria, ensuring satisfactory assay performance prior to reporting clinical results.

All comparator method testing was performed at both KMI Diagnostics (all study cohorts) and DiaSorin S.p.A (a portion of *Active TB* cohort only).

Table 23. Clinical Testing Sites

	Testing Performed at Site
Clinical Study Test SITE 1	• **LIAISON QuantiFERON-TB Gold (clinical testing);** • **QIAGEN QFT-Plus (comparator method testing);** • **5-Day Reproducibility Study**
Clinical Study Test SITE 2	• **LIAISON QuantiFERON-TB Gold (clinical testing);** • **5-Day Reproducibility Study**
Clinical Study Test SITE 3	• **LIAISON QuantiFERON-TB Gold (clinical testing);** • **5-Day Reproducibility Study**
Clinical Study Test SITE 4	• **LIAISON QuantiFERON-TB Gold (clinical testing);** • **QIAGEN QFT-Plus (comparator method testing);** • **20-Day Precision Study;** • **All development activities and all Analytical Studies**

D. Safety and Effectiveness Results

1. Safety Results - There were no adverse events of the device reported at any time during the conduct of the clinical performance study.

2. Effectiveness Results – The clinical performance study results demonstrate the effectiveness of the LIAISON QuantiFERON-TB Gold Plus assay, the LIAISON Control QuantiFERON-TB Gold Plus, and the LIAISON QuantiFERON Software in the

detection of IFN-γ in human heparinized whole blood by chemiluminescence immunoassay using the LIAISON XL Analyzer, in active TB, mixed risk, and low risk, study subject cohorts.

2.1 Clinical Sensitivity – Active TB Cohort

Prospective and retrospective[5] whole blood samples were obtained and processed with the QIAGEN QuantiFERON-TB Gold Plus (QFT-Plus) blood collection tubes from 160 study subjects who presented with signs and symptoms of active TB disease confirmed by culture with either positive AFB smear or nucleic acid amplification test (active TB cohort). Subjects had not received TB treatment or had received less than 14 days of treatment prior to blood collection. All samples were tested with the LIAISON QuantiFERON-TB Gold Plus assay and the QIAGEN QuantiFERON-TB Gold Plus test.

A summary of the clinical sensitivity comparative performance data in terms of obtained versus expected positive results, stratified by sample collection method (i.e., prospective, retrospective), is provided in Table 24 below. The performance results are based on the total number of valid results. Indeterminate (IND) results were not used in the performance calculations. The frequency of indeterminate results for QFT-Plus and the LIAISON QuantiFERON-TB Gold Plus assay was 2.5% (4/160) and 3.8% (6/160), respectively.

Sensitivity of the LIAISON QuantiFERON-TB Gold Plus assay, when testing retrospectively and prospectively collected samples was 96.1% (49/51) and 78.6% (81/103), respectively. The overall sensitivity of the LIAISON QuantiFERON-TB Gold Plus assay was 84.4% (130/154). The sensitivity of the QFT-Plus test, when testing retrospectively collected, and prospectively collected samples was 86.3% (44/51) and 79.0% (83/105), respectively. The overall sensitivity of the QFT-Plus test was 81.4% (127/156).

Table 24. Clinical Sensitivity Study Performance Summary Data

Clinical Sensitivity - Active TB Cohort		Pos	Pos	Neg	Neg	IND	IND	Sensitivity 95% CI	Sensitivity 95% CI
	N	QFT-Plus	Liaison	QFT-Plus	Liaison	QFT-Plus	Liaison	Qiagen QFT-Plus	DiaSorin Liaison
Retrospective†	51	44	49	7	2	0	0	86.3% (44/51) 74.3%-93.2%	96.1% (49/51) 86.8%-98.9%
Prospective‡	109	83	81	22	22	4	6	79.0% (83/105) 70.31% -85.74%	78.6% (81/103) 69.77% - 85.45%
Cumulative Total	160	127	130	29	24	4	6	81.4% (127/156) 74.6%-86.7%	84.4% (130/154) 77.9%-89.3%

†Retrospective samples were obtained from study subjects with active culture confirmed TB; however retrospective samples were chosen for LIAISON QuantiFERON-TB Gold Plus assay testing due to a previous known positive QFT-Plus test result.
‡Prospective samples were obtained from study subjects with active cultured confirmed TB; the QFT-Plus test result was unknown at the time of enrollment.

[5] The active TB cohort included prospectively collected whole blood samples from 109 study subjects, and retrospectively collected whole blood samples from 51 study subjects. Retrospective samples, not unlike the prospective samples, were obtained from study subjects with active culture confirmed TB; however retrospective samples were chosen for LIAISON QuantiFERON-TB Gold Plus assay testing due to a previous QFT-Plus positive test result.

2.2 Clinical Specificity – Low Risk Cohort

Whole blood was prospectively collected and processed with the QIAGEN QuantiFERON-TB Gold Plus (QFT-Plus) blood collection tubes from 312 study subjects with no identified risk factors for TB infection (low risk). All samples were tested with the LIAISON QuantiFERON-TB Gold Plus assay and the QIAGEN QuantiFERON-TB Gold Plus test.

A summary of the clinical specificity comparative performance data, in terms of obtained versus expected negative results is provided in Table 25 below. The performance data are based on the total number of results. Indeterminate (IND) results were not used in the performance calculations. The frequency of indeterminate results for QFT-Plus and the LIAISON QuantiFERON-TB Gold Plus assay was 9.0% (28/312) and 7.7% (24/312), respectively.

Specificity of the LIAISON QuantiFERON-TB Gold Plus assay and the QFT-Plus was 96.9% (279/288) and 97.2% (276/284), respectively.

Table 25. Clinical Specificity Study Performance Summary Data

Clinical Specificity - Low Risk Cohort		Pos	Pos	Neg	Neg	IND	IND	Specificity 95% CI	Specificity 95% CI
	N	QFT-Plus	Liaison	QFT-Plus	Liaison	QFT-Plus	Liaison	Qiagen QFT-Plus	DiaSorin Liaison
Prospective	312	8	9	276	279	28	24	97.2% (276/284) 94.5% - 98.6%	96.9% (279/288) 94.2% - 98.3%

2.3 Clinical Performance - Mixed Risk Cohort

Whole blood was prospectively collected and processed with the QIAGEN QuantiFERON-TB Gold Plus (QFT-Plus) blood collection tubes from 570 healthy study subjects with identified risk factors for LTBI (mixed risk). All samples were tested with the LIAISON QuantiFERON-TB Gold Plus assay and the assay results were compared to the QIAGEN QuantiFERON-TB Gold Plus test results.

A summary of comparative performance data is provided in a 2X2 tabular format in Table 26 below. The performance data were based on the total number of valid results. Two samples with indeterminate (IND) results were not included in the performance calculations.

The comparative performance data revealed a positive percent agreement (PPA) of 96.7% (58/60), 95% CI (88.6 – 99.1), a negative percent agreement (NPA) of 99.4% (505/508), 95% CI (98.3 – 99.8), and an overall agreement of 99.1% (563/568), 95% CI (98.0 – 99.6).

Table 26. Mixed Risk Performance Study Summary Data

Clinical Performance - Mixed Risk Cohort		QFT-Plus		
		Pos (+)	Neg (-)	Total
LIAISON QuantiFERON-TB Gold Plus	Pos (+)	58	3	61
	Neg (-)	2	505	507
Total		60	508	568[†]

[†]Two samples with IND results were not included in the analysis.

2.4 Comparative Clinical Performance Data Summary

The comparative performance of the LIAISON QuantiFERON-TB Gold Plus versus the QFT-Plus for the three clinical study cohorts is illustrated in Table 27 below.

Table 27. Comparative Clinical Performance Data Summary (All Cohorts)

Cohort	N	PPA (95% CI)	NPA (95% CI)	Overall (95% CI)
Active TB	154[A]	98.4% (124/126) (94.4% - 99.6%)	78.6% (22/28) (60.5% - 89.8%)	94.8% (146/154) (90.1% - 97.3%)
Low Risk	278[B]	87.5% (7/8) (52.9% - 97.8%)	99.3% (268/270) (97.3% - 99.8%)	98.9% (275/278) (96.9% - 99.6%)
Mixed Risk	568[C]	96.7% (58/60) (88.6% - 99.1 %)	99.4% (505/508) (98.3% - 99.8%)	99.1% (563/568) (98.0% - 99.6%)
Total	1000[D]	97.4% (189/194) (94.1% - 98.9%)	98.6% (795/806) (97.6% - 99.2%)	98.4% (984/1000) (97.4% - 99.0%)

[A] Total of 6 IND results not included in data analysis

[B] Total of 34 IND results not included in data analysis

[C] Total of 2 IND results not included in data analysis

[D] Cumulative total of 42 IND results not included in analysis

2.5 Comparative Clinical Performance Summary – BCG Vaccinated Study Subjects

A total of 69 study subjects in the active TB cohort and 10 study subjects in the mixed risk study cohort reported having received the BCG TB vaccine. Samples from these study subjects were tested with the LIAISON QuantiFERON-TB Gold Plus assay and results were compared to the QIAGEN QuantiFERON-TB Gold Plus test results. A summary of comparative performance data is provided in a 2X2 tabular format in Table 28 below. The performance data were based on the total number of valid results. Three samples with indeterminate (IND) results were not included in the performance calculations.

The comparative performance data revealed a positive percent agreement (PPA) of 100% (54/54), 95% CI (93.4 – 100), a negative percent agreement (NPA) of 72.7% (16/22), 95% CI (51.8 – 86.8), and an overall agreement of 92.1% (70/76), 95% CI (83.8 – 96.3).

Table 28. Comparative Clinical Performance Summary – BCG Vaccinated Study Subjects

BCG Vaccinated Study Subjects		QFT-Plus		
		Pos (+)	Neg (-)	Total
LIAISON QuantiFERON-TB Gold Plus	Pos (+)	54	6	60
	Neg (-)	0	16	16
Total		54	22	76

2.6 Comparative Clinical Performance Summary – HIV (+) Study Subjects

A total of 13 study subjects in the active TB cohort and 40 study subjects in the mixed risk study cohort reported infection with the HIV. Samples from these study subjects were tested with the LIAISON QuantiFERON-TB Gold Plus assay and results were compared to the QIAGEN QuantiFERON-TB Gold Plus test results. A summary of comparative performance data is provided in a 2X2 tabular format in Table 29 below. The performance data are based on the total number of valid results.

The comparative performance data revealed a positive percent agreement (PPA) of 86% (11/13), 95% CI (57.8 – 95.7), a negative percent agreement (NPA) of 100% (40/40), 95% CI (91.2-100), and an overall agreement of 96.2% (51/53), 95% CI (87.2 – 99.0).

Table 29. Comparative Clinical Performance Summary – HIV (+) Study Subjects

HIV (+) Study Subjects		QFT-Plus		
		Pos (+)	Neg (-)	Total
LIAISON QuantiFERON-TB Gold Plus	Pos (+)	11	0	11
	Neg (-)	2	40	42
Total		13	40	53

2.7 External 5-Day Reproducibility Study

A five-day multi-site reproducibility study was conducted in the United States at three study sites. The study included testing of two lots of LIAISON QuantiFERON-TB Gold Plus assay kits and one lot of the LIAISON Control QuantiFERON-TB Plus kit. The plasma sample test panel used for this study were the same coded samples and controls used in the 20-Day Precision Study (Table 3, above). Each panel member was tested at three study sites, with six replicates per run, and one run per day over a period of five days, resulting in a total of 90 data points per lot (180 data points across two lots). Multiple operators performed

testing at each study site.[6] A summary of the reproducibility study results, stratified by assay kit lot and study site are illustrated in Tables 30 – 33 below.

Table 30. LIAISON® QuantiFERON®-TB Gold Plus Assay Reproducibility Study Summary Results - Combined Study Sites and Assay Kit Lots

Sample ID	N	Mean IU/mL	Repeatability		Between-Day		Between Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Level 1	180	0.068	0.009	12.70%	0.012	17.00%	0.014	20.80%	0.020	29.70%
Ctrl Level 2	180	1.598	0.058	3.70%	0.102	6.40%	0.081	5.00%	0.142	8.90%
QFTB-01-P01	180	0.273	0.011	4.00%	0.023	8.30%	0.013	4.70%	0.034	12.60%
QFTB-01-P02	180	0.329	0.015	4.50%	0.022	6.60%	0.004	1.10%	0.032	9.90%
QFTB-01-P03	180	0.574	0.018	3.20%	0.031	5.50%	0.004	0.70%	0.048	8.40%
QFTB-01-P04	180	0.867	0.031	3.50%	0.046	5.30%	0.025	2.90%	0.070	8.10%
QFTB-01-P05	180	1.691	0.081	4.80%	0.104	6.20%	0.050	3.00%	0.154	9.10%
QFTB-01-P06	180	3.193	0.126	3.90%	0.170	5.30%	0.070	2.20%	0.236	7.40%
QFTB-01-P07	180	4.093	0.131	3.20%	0.188	4.60%	0.122	3.00%	0.265	6.50%
QFTB-01-P08	180	5.606	0.165	3.00%	0.242	4.30%	0.103	1.80%	0.342	6.10%
QFTB-01-P09	180	6.062	0.246	4.10%	0.283	4.70%	0.137	2.30%	0.399	6.60%
QFTB-01-P10	180	6.974	0.199	2.90%	0.347	5.00%	0.142	2.00%	0.446	6.40%

Table 31. LIAISON® QuantiFERON®-TB Gold Plus Assay Reproducibility Study Summary Results – Site 1, Combined Assay Kit Lots

Sample ID	N	Mean (IU/mL)	Repeatability		Between-Day		Between Lot		Total Within-Site	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Level 1	60	0.074	0.006	8.30%	0.009	11.90%	0.017	22.90%	0.020	27.10%
Ctrl Level 2	60	1.611	0.041	2.50%	0.095	5.90%	0.084	5.20%	0.133	8.30%
QFTB-01-P01	60	0.286	0.008	2.80%	0.019	6.70%	0.005	1.90%	0.021	7.50%
QFTB-01-P02	60	0.341	0.010	3.00%	0.014	4.30%	0.000	0.00%	0.018	5.20%
QFTB-01-P03	60	0.602	0.015	2.60%	0.034	5.60%	0.000	0.00%	0.037	6.10%
QFTB-01-P04	60	0.899	0.021	2.30%	0.037	4.10%	0.000	0.00%	0.042	4.70%
QFTB-01-P05	60	1.756	0.065	3.70%	0.081	4.60%	0.000	0.00%	0.104	5.90%
QFTB-01-P06	60	3.248	0.088	2.70%	0.134	4.10%	0.082	2.50%	0.180	5.50%
QFTB-01-P07	60	4.188	0.090	2.10%	0.130	3.10%	0.118	2.80%	0.197	4.70%
QFTB-01-P08	60	5.753	0.147	2.60%	0.169	2.90%	0.086	1.50%	0.240	4.20%
QFTB-01-P09	60	6.173	0.201	3.30%	0.215	3.50%	0.111	1.80%	0.315	5.10%
QFTB-01-P10	60	7.116	0.143	2.00%	0.313	4.40%	0.035	0.50%	0.346	4.90%

[6] The number of operators that performed testing at each study site: Site1 = 4 operators, Site2 = 2 operators, Site3 = 3 operators.

Table 32. Reproducibility Study Summary Results – Site 2, Combined Assay Kit Lots

Sample ID	N	Mean (IU/mL)	Repeatability		Between-Day		Between Lot		Total Within-Site	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Level 1	60	0.059	0.006	9.50%	0.008	13.80%	0.014	23.80%	0.017	29.10%
Ctrl Level 2	60	1.546	0.055	3.60%	0.134	8.60%	0.034	2.20%	0.149	9.60%
QFTB-01-P01	60	0.247	0.010	4.00%	0.021	8.40%	0.000	0.00%	0.023	9.30%
QFTB-01-P02	60	0.306	0.020	6.40%	0.025	8.10%	0.000	0.00%	0.032	10.30%
QFTB-01-P03	60	0.538	0.021	3.90%	0.035	6.50%	0.000	0.00%	0.041	7.60%
QFTB-01-P04	60	0.820	0.035	4.20%	0.059	7.20%	0.000	0.00%	0.068	8.30%
QFTB-01-P05	60	1.603	0.089	5.50%	0.136	8.50%	0.000	0.00%	0.163	10.10%
QFTB-01-P06	60	3.068	0.147	4.80%	0.238	7.80%	0.097	3.20%	0.296	9.70%
QFTB-01-P07	60	3.959	0.146	3.70%	0.292	7.40%	0.165	4.20%	0.366	9.20%
QFTB-01-P08	60	5.408	0.141	2.60%	0.332	6.10%	0.181	3.40%	0.404	7.50%
QFTB-01-P09	60	5.965	0.293	4.90%	0.432	7.20%	0.263	4.40%	0.585	9.80%
QFTB-01-P10	60	6.742	0.188	2.80%	0.466	6.90%	0.201	3.00%	0.541	8.00%

Table 33. Reproducibility Study Summary Results – Site 3, Combined Assay Kit Lots

Sample ID	N	Mean (IU/mL)	Repeatability		Between-Day		Between Lot		Total Within-Site	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ctrl Level 1	60	0.072	0.012	17.30%	0.016	22.50%	0.018	24.30%	0.027	37.40%
Ctrl Level 2	60	1.638	0.074	4.50%	0.066	4.00%	0.140	8.60%	0.172	10.50%
QFTB-01-P01	60	0.285	0.014	4.90%	0.027	9.60%	0.022	7.70%	0.038	13.30%
QFTB-01-P02	60	0.340	0.013	3.90%	0.022	6.50%	0.015	4.40%	0.030	8.80%
QFTB-01-P03	60	0.582	0.018	3.20%	0.021	3.60%	0.019	3.20%	0.033	5.80%
QFTB-01-P04	60	0.881	0.034	3.90%	0.033	3.70%	0.053	6.00%	0.071	8.10%
QFTB-01-P05	60	1.714	0.086	5.00%	0.068	4.00%	0.112	6.50%	0.157	9.10%
QFTB-01-P06	60	3.263	0.134	4.10%	0.108	3.30%	0.000	0.00%	0.172	5.30%
QFTB-01-P07	60	4.131	0.148	3.60%	0.058	1.40%	0.060	1.50%	0.170	4.10%
QFTB-01-P08	60	5.657	0.202	3.60%	0.179	3.20%	0.000	0.00%	0.270	4.80%
QFTB-01-P09	60	6.048	0.234	3.90%	0.085	1.40%	0.072	1.20%	0.259	4.30%
QFTB-01-P10	60	7.065	0.251	3.60%	0.217	3.10%	0.139	2.00%	0.359	5.10%

3. Subgroup Analyses
   Not Applicable.

4. Pediatric Extrapolation – In this premarket application, clinical data was not leveraged to support approval of a claim for use in pediatric patient populations.

E. Financial Disclosure

The *Financial Disclosure by Clinical Investigators* regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The clinical performance study included four investigators (Table 23). None of the clinical investigators had disclosable financial interests/arrangements as defined in Sections 54.2(a), (b), (c), and, (f). The information provided does not raise any questions about the reliability of the study data.

XI. PANEL MEETING RECOMMENDATION

In accordance with the provisions of Section 513(c)(3) of the act as amended by the Safe Medical Devise Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM ANALYTICAL AND CLINICAL PERFORMANCE STUDIES

A. Effectiveness Conclusions

1. The effectiveness of the LIAISON QuantiFERON-TB Gold Plus assay, the LIAISON Control QuantiFERON-TB Gold Plus, and the LIAISON QuantiFERON Software, in the detection of IFN-γ in human heparinized whole blood by chemiluminescence immunoassay using the LIAISON XL Analyzer has been adequately demonstrated.
2. No interference in the performance of the LIAISON QuantiFERON-TB Gold Plus assay or the LIAISON Control QuantiFERON-TB Gold Plus was observed with endogenous substances, medications, and biotin (Section B).
3. Harvested plasma samples for LIAISON QuantiFERON-TB Gold Plus assay testing can be stored at 2-8°C for 28 days or at -20°C for six months. Frozen samples are stable for up to four freeze/thaw cycles (Section F).
4. The LIAISON QuantiFERON-TB Gold Plus assay kit shelf life, based on real-time study data, is currently 13 months after date of manufacture when stored at 2-8°C. The LIAISON Control QuantiFERON TB Gold Plus kit shelf life, based on real-time study data, is currently 13 months after date of manufacture when stored at 2-8°C. The assay kit and control kit long term stability (shelf life) study protocols have been reviewed and are acceptable (Section H).
5. The LIAISON QuantiFERON-TB Gold Plus Reagent Integral is stable, once opened, for four weeks when stored on board the LIAISON XL Analyzer (Section I.2).
6. The LIAISON QuantiFERON-TB Gold Plus calibrators are stable, once opened and reconstituted, for four weeks when stored at 2-8°C (Section I.3).
7. The LIAISON QuantiFERON-TB Gold Plus conjugate is stable, once opened and reconstituted, for four weeks when stored at 2-8°C (Section I.1).

8. The LIAISON Control QuantiFERON-TB Gold Plus (Level 1 and Level 2) are stable, once opened and reconstituted, for four weeks when stored at 2-8°C (Section J).
9. Calibration of the LIAISON QuantiFERON-TB Gold Plus assay is stable for four weeks (Section G).

B. Safety Conclusions

Based on the results of the analytical and clinical performance studies the LIAISON QuantiFERON-TB Gold Plus assay, the LIAISON Control QuantiFERON-TB Gold Plus, and the LIAISON QuantiFERON Software, when used in accordance with the accompanying instructions for use, and in conjunction with risks assessment, radiography, and other medical and diagnostic evaluations, should be safe and should pose minimal risk to the patient due to inaccurate test results.

C. Benefit-Risk Determination

The probable benefits and risks of the device are based on data collected in the clinical study conducted to support PMA approval described above. The primary benefit of the assay is the determination of *M. tuberculosis* infection, which will assist clinicians in making individual patient management decisions, including initiation of appropriate monitoring and antimicrobial therapy. Diagnosis and treatment of latent or active *M. tuberculosis* infection in appropriate patients can prevent the sequelae of disease and may result in reduced morbidity and mortality in these patients as well as decreased reactivation or relapse of disease and development of drug resistance. Additionally, diagnosis and appropriate treatment can potentially reduce transmission and disease burden in the general population as well as in populations at high risk for *M. tuberculosis* infection. Accurate diagnosis of *M. tuberculosis* infection also may lead clinicians to evaluate and subsequently treat patients for human immunodeficiency virus (HIV) as management of *M. tuberculosis* infection can depend on HIV status.

The probable risks associated with the device are those related to the risk of erroneous test results, failure to correctly interpret the test results, and failure to correctly operate the instrument. The risks of a false positive test include improper patient management, such as unnecessary antimicrobial treatment or infection control measures. Antimicrobial therapy requires frequent follow-up and patients may develop side-effects, namely hepatotoxicity, visual acuity deficits, and less often GI disturbance, drug-drug interactions, renal dysfunction and associated electrolyte disturbances, thyroid dysregulation, mood changes, peripheral neuropathy, retinitis, bone marrow suppression, arrhythmias, ototoxicity, and allergic/drug reactions. Patients with high clinical suspicion for active tuberculosis will require other diagnostic tests and may be diagnosed via other means. The risks of a false positive is mitigated by the acceptable performance characteristics in patients with risk factors for TB, in whom the test will most likely be used to diagnose latent tuberculosis, as those patients are most likely to benefit from testing.

The risk of a false negative test includes potentially failing to treat a patient who has tuberculosis. Patients with high clinical suspicion for active tuberculosis may be diagnosed via other means, reducing risk of a false negative in this population. The risk of a false negative test result in patients being evaluated for latent tuberculosis include a missed opportunity to provide preventative antimicrobial therapy to reduce the risk of developing active tuberculosis disease. The favorable performance characteristics of the assay compared to an appropriate currently-marketed comparator suggests that this assay will not add significant risk to current medical practice.

Additional factors to be considered in determining probable risks and benefits for the LIAISON QuantiFERON-TB Gold Plus assay included:

- Uncertainty due to reporter bias and wide confidence intervals that are expected due to the small sample sizes in subgroup analysis.
- Uncertainty of the benefits due to the imperfect comparator method used to calculate performance characteristics.
- Eligibility for inclusion in the clinical trial was based on case report forms for the subject groups. Inclusion based on case report forms is common practice in clinical trials. Reporter and recall bias may incur an acceptable amount of expected uncertainty pertaining to the inclusion and exclusion of subjects and thus interpretation of data.

This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for indirect testing for *Mycobacterium tuberculosis* infection (including disease) when used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this premarket application support a reasonable assurance of safety and effectiveness of the LIAISON QuantiFERON-TB Gold Plus assay, the LIAISON Control QuantiFERON-TB Gold Plus, and the LIAISON QuantiFERON Software, when used in accordance with the indications for use and the instructions for use in the device labeling.

XIII. CDRH DECISION

CDRH issued an approval order on November 26, 2019.

The sponsor's manufacturing facilities have be inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for Use: See device labeling.

Hazards to Health due to Use of the Device: See Indications for Use (Section II), Contraindications (Section III), Warnings and Precautions (Section IV), and Adverse Events (Section X.D)

Post-Approval Requirements and Restrictions: See Approval Order