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CE 0459

Changes: §13;
Deletions: §14;

LIAISON® QuantiFERON®-TB Gold Plus (REF 311050)

1. INTENDED PURPOSE

The DiaSorin LIAISON® QuantiFERON®-TB Gold Plus assay uses chemiluminescent immunoassay (CLIA) technology for the detection of interferon-γ (IFN-γ) in human lithium heparin plasma specimens. The immunoassay can identify *in vitro* responses to a peptide antigens cocktail associated with Mycobacterium tuberculosis (M. tuberculosis) infection (including disease) that stimulates cells in heparinized whole blood collected with the QuantiFERON®-TB Gold Plus Blood Collection Tubes. The assay is an indirect test intended as an aid in the diagnosis of M. tuberculosis infection. Although the assay quantitatively detects the IFN-γ, the interpretation of the result for a single patient is strictly qualitative. The assay must be performed on the LIAISON® XL and LIAISON® XS analyzers only.

2. SUMMARY AND EXPLANATION OF THE TEST

Tuberculosis (TB) is a communicable disease, transmitted almost exclusively by cough aerosols carrying pathogens of the *M. tuberculosis* complex. TB continues to be a major public health threat, causing an estimated 10 million new cases, with 1.4 million deaths from TB in 2019[1]. Pathogenesis is characterized by a period of asymptomatic subclinical infection, defined broadly as latent tuberculosis infection (LTBI), which might last for weeks or decades. However, there is no diagnostic gold standard for LTBI. Two tests are available for the identification of LTBI: the tuberculin skin test (TST) and the interferon gamma release assay (IGRA). They represent indirect markers of *M. tuberculosis* exposure and indicate a cellular immune response to *M. tuberculosis*.

From an operational point of view, LTBI may best be defined as a state of persistent immune response to *M. tuberculosis* antigens detected either by the TST or by IGRA without evidence of clinically manifest TB. Based on this definition, individuals with LTBI carry an increased risk of progression to TB. However, an unknown but large number of those with LTBI will not develop TB, either because their immune system persistently controls mycobacterial replication or because they are no longer infected with live bacteria[2].

In most individuals, initial *M. tuberculosis* infection is eliminated or contained by the host's defenses, and infection remains latent. However, latent TB bacilli may remain viable and "reactivate" later in life, causing active TB disease. Identification and treatment of LTBI can substantially reduce the risk of developing active disease.

The goal of testing for LTBI is to identify individuals who are at increased risk of developing active TB; these individuals would benefit most from treatment of LTBI (also termed preventive therapy or prophylaxis).

In general, testing for LTBI is indicated when there is a higher risk of developing disease from latent infection (if present); examples include likely recent infection (e.g., close contact of a person with TB) or a decreased capacity to contain latent infection (e.g., because of immunosuppression, as in the case of young children who come in contact with people with active TB, people living with human immunodeficiency virus [HIV] infection or persons who are immunosuppressed because of medications or conditions such as uncontrolled diabetes).

The TST, performed using the Mantoux technique[3], consists of an intradermal injection of a purified protein derivative (PPD). In a person who has cell-mediated immunity to these tuberculin antigens, a delayed-type hypersensitivity reaction will occur within 48 to 72 h. The reaction will cause localized induration of the skin at the injection site, and the transverse diameter should be measured (as millimeters of induration) by a trained individual and interpreted using risk-stratified cutoffs[4]. It is important to note that cell-mediated immunity to tuberculin antigens can sometimes reflect exposure to similar antigens from environmental *Mycobacteria* or *M. bovis bacillus Calmette-Guérin* (BCG) vaccination or a previous infection that has been cleared (through immunological mechanisms or treatment). The TST has several known limitations. Completing the TST requires two health care visits, for tuberculin injection and induration measurement, which results in loss of reading in approximately 10% of cases[5]. In addition, measurement of reaction size is subject to inter-observer variability, although this is greatly reduced with adequate training[6]. False-positive and false-negative results may occur. There are two important causes of false-positive results: *non tuberculous Mycobacteria* (NTM) infection and prior BCG vaccination[7]. False-negative TST results may occur because of limited sensitivity in particular patient subgroups (e.g., immunosuppressed individuals, due to medical conditions such as HIV infection or malnutrition, or those taking immunosuppressive medications), due to severe tuberculosis disease or because of pre-analytical or analytical sources of test variability (e.g., improper tuberculin handling or placement or incorrect interpretation of test results)[8].

IGRAs are *in vitro* blood tests of cell-mediated immune response; they measure T-cell release of interferon-γ (IFN-γ) following stimulation by antigens specific to the *M. tuberculosis complex* (with the exception of BCG substrains), i.e., early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are encoded by genes located within the region of difference 1 (RD1) locus of the *M. tuberculosis* genome[9,10]. They are more specific than PPD for M. tuberculosis because they are not encoded in the genomes of any BCG vaccine strains or most species of NTM, other than *M. marinum*, *M. kansasii*, *M. szulgai*, and *M. flavescens*[11]. However, not all NTMs have been studied for cross-reactivity. There is some evidence of cross-reactivity between ESAT-6 and CFP-10 of *M. tuberculosis* and *M. leprae*[12,13], but the clinical significance of this in settings where leprosy and TB are endemic (e.g., India and Brazil) is poorly characterized. IGRAs appear to be unaffected by most infections with NTM, which can cause false positive TSTs[14].

Since there is no gold standard for LTBI, sensitivity and specificity are typically estimated using surrogate reference standards. Sensitivity is estimated among culture-confirmed TB cases, while specificity is estimated among low-risk individuals with no known TB exposure in low-incidence settings[15].

The TST and IGRA tests are based on immunological sensitization to mycobacterial antigens; a practical benefit of IGRA tests is that they require only a single laboratory test with negative and positive controls, and only one visit. By nature, functional T-cell assays are highly susceptible to variability by numerous factors at multiple levels, including assay manufacturing, pre-analytical processing, analytical testing and immunomodulation, therefore cases of conversion and reversion in individuals undergoing serial testing should not be excluded.

Among the IGRAs, QuantiFERON®-TB Gold Plus is an ELISA-based IFN-γ detection assay developed and marketed by QIAGEN and based on the incubation of human whole blood samples in specific QuantiFERON®-TB Gold Plus Blood Collection Tubes coated with specific *M. tuberculosis* antigens eliciting the patient's T-cells immunized to *M. tuberculosis* to secrete cytokines including IFN-γ.

In September 2018, according to a co-development project with QIAGEN, DiaSorin introduced in the CE Market a chemiluminescent immunoassay (CLIA) version of QuantiFERON®-TB Gold Plus to be used on the LIAISON® XL automated platforms and based on the same principles; in October 2019, the assay was released in the CE market also on the LIAISON® XS CLIA platform.

The method used to determine IFN-γ is a direct, sandwich CLIA. Monoclonal antibodies to IFN-γ (mouse monoclonal) are used for coating magnetic particles (solid phase) and monoclonal antibodies to IFN-γ (mouse monoclonal) are linked to an isoluminol derivative (isoluminol-antibody conjugate): binding between monoclonal antibodies and isoluminol in the conjugate is mediated by a Biotin-Streptavidin immuno-complex.

During the first incubation, IFN-γ present in calibrators, samples or controls will bind to the solid phase and Conjugate and form a sandwich. During the second incubation, Assay Buffer W is added. It reduces non-specific, sample-related bindings. After each incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescent reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the IFN-γ concentration present in calibrators, samples or controls.

3. PRINCIPLE OF THE PROCEDURE

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4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles (≥0.25% solid) coated with antibody to human IFN-γ (mouse monoclonal) (approx. 250 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Conjugate (3.2 mL)	CONJ	Biotinylated Antibody to human IFN-γ (mouse monoclonal) (approx. 3.75 µg/mL) linked to Streptavidin-MPABEI conjugate (approx. 4.5 µg/mL), phosphate buffer, EDTA, BSA, 0.2% ProClin™ 300, gentamicin sulfate 0.1 g/L, detergents.
Diluent (18 mL)	DIL	BSA, casein, phosphate buffer, EDTA, 0.2% ProClin™ 300, non-specific IgG (mouse polyclonal), gentamicin sulphate 0.1 g/L.
Assay Buffer W (2 x 23 mL)	BUF W	BSA, casein, phosphate buffer, EDTA, 0.2% ProClin™ 300 and an inert blue dye.
Number of tests		200

The order of reagents reflects the layout of containers in the reagent integral.

Included in the kit

Calibrator A (lyophilized, 2 mL)	CAL A	Recombinant human IFN-γ (produced in *E. coli*) (approx. 0.47 IU/mL), HEPES buffer, BSA, bovine serum, 0.4% ProClin™ 300, 0.2 g/L gentamicin sulfate, detergents. The calibrator concentration (IU/mL) is referenced to WHO International Standard NR-3086.
Calibrator B (lyophilized, 2 mL)	CAL B	Recombinant human IFN-γ (produced in *E. coli*) (approx. 7.2 IU/mL), HEPES buffer, BSA, bovine serum, 0.4% ProClin™ 300, 0.2 g/L gentamicin sulfate, detergents. The calibrator concentration (IU/mL) is referenced to WHO International Standard NR-3086.
1 Barcoded label for reconstituted Calibrator A.		
1 Barcoded label for reconstituted Calibrator B.		

Conjugates, Diluent, Assay Buffer W and Magnetic particles are provided ready-to-use.

Calibrators are provided lyophilized.

Materials required but not provided

LIAISON® XL Analyzer	LIAISON® XS Analyzer
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055). LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300). LIAISON® Wash/System Liquid (REF 319100). – LIAISON® XL Waste Bags (REF X0025). –	LIAISON® Cuvettes on Tray (REF X0053). – LIAISON® Disposable Tips (REF X0055). – LIAISON® EASY Starter Kit (REF 319300). LIAISON® EASY Wash Buffer (REF 319301). LIAISON® EASY System Liquid (REF 319302). LIAISON® EASY Waste (REF X0054). LIAISON® EASY Cleaning Tool (REF 310996).

Additionally required materials

LIAISON® Control QuantiFERON®-TB Gold Plus (REF 311051).

Additionally required materials from other suppliers

QuantiFERON®-TB Gold Plus Blood Collection Tubes (REF 622222, 622423, 623222, 622526, 623526, 623423) (for orders and information contact a QIAGEN local representative or visit www.qiagen.com).

Additionally available tool

LIAISON® QuantiFERON® Software (LQS) (REF Q0001).
LIAISON® ASYc (software for qualitative interpretation of results) (REF ASY-C).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

For Laboratory Professional Use Only.

Visually inspect the integral vials for leakage at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

All materials used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. Waste must be handled with care and disposed of in compliance with laboratory guidelines and the statutory provisions in force in each Country.

Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

The LIAISON® XL and LIAISON® XS analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.

Do not mix reagents from different reagent packs (even for the same reagent).

Do not use kits or components after the expiration date given on the label.

Strict adherence to the instructions is necessary to obtain reliable results.

Pursuant to EC Regulation 1272/2008 (CLP), hazardous reagents are classified and labeled as follows:

REAGENTS:	BUF W, DIL, CONJ	CAL A (lyophilized), CAL B (lyophilized)
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Eye irrit. 2 H319 Skin irrit. 2 H315 Skin sens. 1A H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	  GHS07 Exclamation mark GHS09 Environment
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H315 Causes skin irritation. H317 May cause an allergic skin reaction. H319 Causes serious eye irritation. H410 Very toxic to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/ vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/ eye protection/face protection. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P273 Avoid release to the environment. P391 Collect spillage.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300). gentamicin sulfate salt.

Note: after reconstitution, CAL A, CAL B are classified as indicated below:

REAGENTS:	CAL A (reconstituted), CAL B (reconstituted)
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labelled as EUH210, safety data sheets available on request.

For additional information, see the Safety Data Sheets available on www.diasorin.com.

7. PREPARATION OF REAGENTS

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

Before the seal is removed, rotate the small wheel in the magnetic particle compartment until the color of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Carefully wipe the surface of each septum to remove residual liquid.

Repeat as necessary until the magnetic particles are completely resuspended.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

Visually inspect the reagents, and the calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foaming is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of the integral into the reagent area

– LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device that aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
– Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using it. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

CALIBRATORS

Calibrators for the LIAISON® QuantiFERON®-TB Gold Plus assay are supplied lyophilized. Calibrators are kit lot specific and must be used only with the reagent integral lot they are matched with. Correct lot matching between the reagent integral and Calibrators is reported on the integral label.

Do not pool the contents of different Calibrator vials, even if they belong to the same lot.

Proper reconstitution of Calibrators is essential.

– LIAISON® QuantiFERON®-TB Gold Plus Calibrators are supplied lyophilized.
– Reconstitute the vial contents with 2.0 mL of deionized or distilled water.
– Allow the vials to stand for at least 15 minutes at 18-25°C to achieve complete dissolution.
– Affix the appropriate, additionally provided barcode label to the vial.
– Mix vials thoroughly by gentle inversion; avoid foaming.
– The reconstituted solution of each calibrator can be stored in original vials and loaded on the instrument on a suitable rack.
– Once reconstituted, refer to paragraph 8 in order to store the calibrators.
– For details on the use of the Calibrators on board the instrument, refer to the analyzer Operator’s Manual.

Original vial labels refer only to lyophilized Calibrators. Once reconstituted, pursuant to EC Regulation 1272/2008 (CLP), calibrators are classified Skin sens. 1A H317 and Aquatic chronic 3 H412. For more details, refer to paragraph 6.

CONTROLS

Refer to the instructions for use of LIAISON® Control QuantiFERON®-TB Gold Plus for proper preparation and handling instructions.

8. REAGENTS STORAGE AND STABILITY

REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles. Refer to Reagent Integral Preparation for resuspension instructions.

– **Sealed**: stable at 2-8°C until the expiry date.
– **Opened at 2-8°C or on board the analyzer**: stable for four (4) weeks.
  Always use the same analyzer for a reagent integral that has already been opened.
– Use the storage rack provided with the analyzer for upright storage of the reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate later proper resuspension of the magnetic particles.
– Keep away from direct light.

CALIBRATORS

– **Lyophilized**: stable at 2-8°C until the expiry date. Upon receipt, the calibrators must be stored at 2-8°C in an upright position to prevent adherence of the lyophilizate to the vial cap.
– **Reconstituted**: stable for four (4) weeks when properly stored at 2-8°C between two successive uses, in their capped vials. After reconstitution, the calibrators must be stored at 2-8°C in an upright position to prevent adherence of the solution to the vial cap.
– Each vial allows 4 calibrations to be performed.

Do not leave the reconstituted calibrators at room temperature longer than the time required to process them on the analyzer. Do not freeze.

During handling, use appropriate precautions to avoid bacterial contamination of calibrators.

9. SPECIMEN COLLECTION AND PREPARATION

LIAISON® QuantiFERON®-TB Gold Plus is validated only with whole blood specimens collected, handled and processed with QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes.

Whole blood shall be collected referring to the instructions for use of the QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes and processed accordingly.

Before loading on the instrument for IFN-γ detection, specimens must be visually inspected: samples having particulate matter, turbidity or erythrocyte debris may require transfer to a secondary tube and additional centrifugation before testing. Lipaemic samples as well as samples exhibiting obvious microbial contamination should not be tested. After incubation and centrifugation of the primary tube some hemolysis may appear. Hemoglobin is not expected to interfere with testing up to 1000 mg/dL. Check for and remove any air bubbles and fibrin clots before assaying.

The test can be performed either on processed and centrifuged QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes or on plasma specimens collected in secondary tubes after centrifugation, loaded on appropriate racks on the LIAISON® XL Analyzer and LIAISON® XS Analyzer (refer to the user manual for additional details).

Proper sample handling is crucial to ensuring the integrity of the sample. For tests directly performed on QIAGEN's tubes, refer to the instructions for use of the QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes. Specimens harvested in secondary tubes can be tested within 28 days if stored at 2-8°C or after an extended period of time if stored deep-frozen (-20°C or below). If samples are stored frozen, mix the thawed samples well before testing. Up to 3 freeze/thaw cycles are allowed for the collected specimens. Thawed samples may require clarification by centrifugation before testing.

The minimum plasma volume required is 210 µL specimen (60 µL specimen + 150 µL dead volume).

10. CALIBRATION

Testing of assay specific calibrators allows the detected relative light unit (RLU) values to adjust to the assigned master curve. Each calibration solution allows four (4) calibrations to be performed.

Calibrators must be used only with the Reagent Integral lot they are matched with. Do not use calibrators matched with a different Reagent Integral lot in the same assay. For correct lot matching, the calibrator lot number is also printed on the Reagent Integral Label.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occur:

– A new Reagent Integral lot is used.
– A new Starter Kit lot is used.
– The previous calibration was performed more than four (4) weeks before.
– **LIAISON® XL Analyzer:** the analyzer has been serviced.
– **LIAISON® XS Analyzer**: after a technical intervention, only if required by the service procedure, as communicated by DiaSorin Technical support or representative.
– The values of the controls lie outside the expected ranges.

Refer to the analyzer Operator's Manual or analyzer Quick Guide for calibration instructions.

LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). If the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support team for instructions.

The analyzer operations are as follows:

1. Dispense diluent, magnetic particles and Conjugate into the reaction module.
2. Dispense calibrators, controls or specimens into the reaction module.
3. Incubate.
4. Wash with Wash/System liquid.
5. Dispense Assay Buffer W.
6. Incubate.
7. Wash with Wash/System liquid.
8. Add the Starter Kit and measure the light emitted.

12. QUALITY CONTROL

LIAISON® Controls should be run in singlicate to monitor assay performance. Quality control must be performed by running LIAISON® Control QuantiFERON®-TB Gold Plus (REF 311051):

(a) at least once per day of use, before running the test;
(b) whenever a new reagent integral is used;
(c) whenever the kit is calibrated;
(d) whenever a new lot of Starter Reagents is used;
(e) in agreement with the guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before being used. Appropriate value ranges should then be established for the quality control materials used.

13. INTERPRETATION OF RESULTS

LIAISON® QuantiFERON®-TB Gold Plus results are interpreted using the following criteria (Table 1) or flowchart (Figure 1). The test sample results are reported in International Units per mL (IU/mL). Although the assay quantitatively detects the IFN-γ, the interpretation of the result for a single patient is strictly qualitative. The magnitude of the measured IFN-γ level cannot be correlated to the stage or degree of infection, level of immune responsiveness or likelihood for progression to active disease.

Assay range: up to 10 IU/mL value of IFN-γ.

Responses to the Mitogen positive control, and occasionally to TB1 and TB2 tubes, can be above the assay range. Do not dilute over-range specimens. Cases of undetectable response might be observed. This does not affect the test results.

For calculation purposes:

IFN-γ values > 10 IU/mL should be handled as 10 IU/mL.

IFN-γ values < 0 IU/mL should be handled as 0 IU/mL.

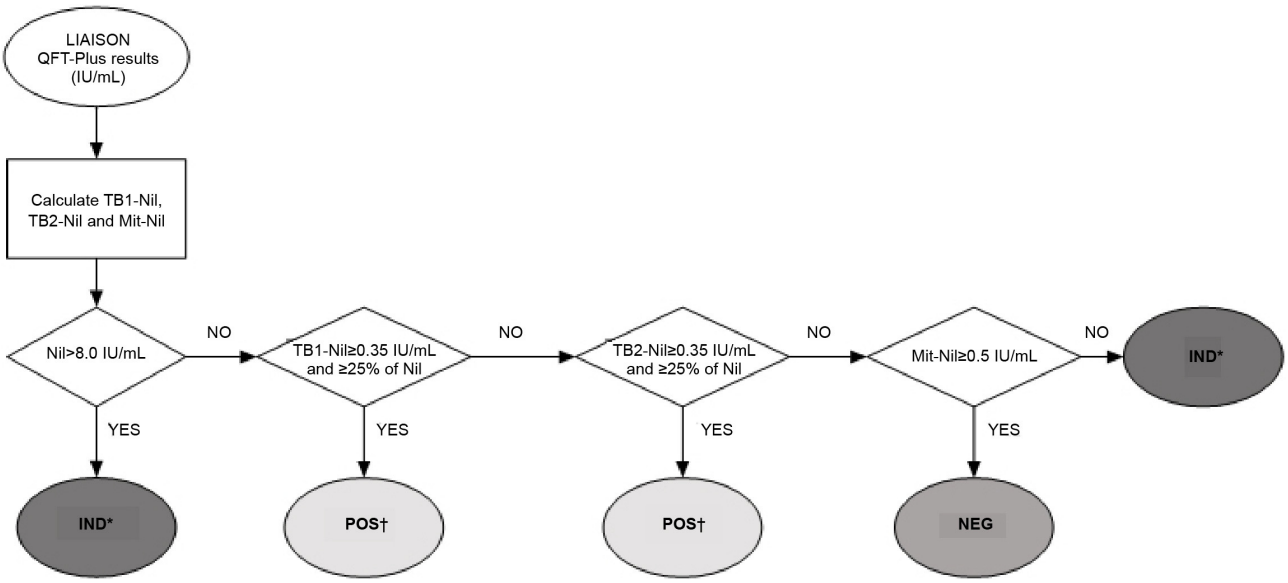
Table 1. Interpretation of LIAISON® QuantiFERON®-TB Gold Plus Assay Results

Nil (IU/mL)	TB1 minus Nil (IU/mL)	TB2 minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	LIAISON® QuantiFERON®-TB Gold Plus result	Report/ Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% of Nil	Any	Any	Positive	M. tuberculosis infection likely
	Any	≥ 0.35 and ≥ 25% of Nil			
	< 0.35 OR ≥ 0.35 and < 25% of Nil	< 0.35 OR ≥ 0.35 and < 25% of Nil	≥ 0.5	Negative	M. tuberculosis infection NOT likely
	< 0.35 OR ≥ 0.35 and < 25% of Nil	< 0.35 OR ≥ 0.35 and < 25% of Nil	< 0.5	Indeterminate	Likelihood of M. tuberculosis infection cannot be determined
> 8.0	Any				

These calculations can be performed also using LIAISON® QuantiFERON® Software (LQS), which is an optional tool provided as an aid for managing the QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes and to interpret LIAISON® QuantiFERON®-TB Gold Plus results or LIAISON® ASYc, which is an optional tool provided as an aid to interpret LIAISON® QuantiFERON®-TB Gold Plus results. Contact your local DiaSorin Sales representative for more information.

In order to obtain correct matching of results among tubes and their interpretation, it is recommended not to set any conversion factor on the analyzer.

Figure 1. Interpretation of LIAISON® QuantiFERON-TB® Gold Plus Assay Results



†Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate. If repeat testing of one or both replicates is positive, the test result is considered positive.

*Indeterminate results are uncommon and may be related to the status of the immune system of the patient. An indeterminate result may also be related to technical factors (e.g., inappropriate storage or handling of the blood collection tubes) if the instructions for use are not followed. If technical issues are suspected with the reagent storage, blood collection or handling of the blood samples, repeat the test with new blood samples. Physicians may choose to redraw a specimen or perform other procedures as appropriate.

14. LIMITATIONS OF THE PROCEDURE AND WARNINGS

1. To obtain a correct interpretation for a patient, combine only the results from tubes collected from the subject in the same sampling session.
2. Results obtained for the same patient from valid runs can be combined, even if assayed with different kit lots and/or on different LIAISON® XL or LIAISON® XS analyzers.
3. Four individual specimen results of the same patient can be combined for the final qualitative interpretation only if the last result is obtained within eighteen (18) hours from the first result and with maximum stability of the sample.
4. In the unlikely event that a subject's result is reported as positive and its Mitogen minus Nil result is less than 0.5 IU/mL, a false-positive result may occur due to a mix-up of the TB antigen and Mitogen samples. Before reporting this positive result, make sure that TB antigen and Mitogen samples have not been mixed up. In this case, LQS and LIAISON® ASYc flag the result as a possible sample mix-up using the "*" symbol.
5. A negative result does not preclude the possibility of M. tuberculosis infection or tuberculosis disease: false-negative results can be due to the stage of infection (e.g., specimen obtained prior to the development of cellular immune response), co-morbid conditions that affect immune functions, incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay or other immunological variables.
6. A positive result should not be the sole or definitive basis for determining infection with M. tuberculosis. Incorrect performance of the assay may cause false-positive responses.
7. A positive result should be followed by further medical evaluation and diagnostic evaluation for active tuberculosis disease (e.g., AFB smear and culture, chest X-ray).
8. While ESAT-6 and CFP-10 are absent from all BCG strains and from most known nontuberculous Mycobacteria, it is possible that a positive result may be due to infection by M. kansasii, M. szulgai or M. marinum. If such infections are suspected, alternative tests should be investigated.
9. Assay results should be utilized in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations to assist the clinician in making individual patient management decisions.
10. Test results are reported in IU/mL for IFN-γ detection, but the interpretation of the patient is strictly qualitative.
11. A skillful technique and strict adherence to the instructions of the QIAGEN QuantiFERON®-TB Gold Plus Blood Collection Tubes and of the immunoassay are necessary to obtain reliable results.
12. Grossly hemolyzed, icteric or lipemic samples, as well as samples containing particulate matter or exhibiting obvious microbial contamination, are not recommended and should not be tested.
13. Bacterial contamination or heat inactivation of the specimens may affect the test results.
14. Heterophilic antibodies in human specimens can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and their results should be evaluated with care.
15. Integrals may not be exchanged between analyzer types (LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Potential interfering substances

Controlled studies of potentially interfering substances at five IFN-γ levels showed no interference at the concentration for each substance listed below in the LIAISON® QuantiFERON®-TB Gold Plus assay. The testing was based on CLSI document EP07.

Substances	Tested concentration	Substances	Tested concentration
Triglycerides	3000 mg/dL	IL-2	10 ng/mL
Hemoglobin	1000 mg/dL	IL-4	5 ng/mL
Unconjugated bilirubin	20 mg/dL	IL-5	100 ng/mL
Conjugated bilirubin	20 mg/dL	IL-6	100 ng/mL
Total protein (high)	120 g/L	IL-10	100 ng/mL
Total protein (low)	38 g/L	IL-12	100 ng/mL
RF (Rheumatoid Factor)	469 IU/mL	IFN-alpha	50 ng/mL
HAMA	600 ng/mL	IFN-beta	50 ng/mL
Cholesterol	350 mg/dL	TNF-alpha	5 ng/mL
Prednisolone	0.3 mg/dL	Biotin	3500 ng/mL
Cyclosporine	5 µg/mL	Abacavir sulfate	15 µg/mL

15.2. Precision with LIAISON® XL Analyzer

A within-laboratory precision study was performed by consulting CLSI document EP05-A3 in the preparation of the testing protocol. Ten (10) Li-Heparin samples containing spiked concentrations of native IFN-γ that span the assay range were assayed in duplicate, in 2 runs per day, over the course of 20 operating days by multiple technicians, using two assay lots. The study was repeated on two LIAISON® XL analyzers, with the same testing protocol. The %CV and minimum and maximum experimental levels of IFN-γ obtained are reported in the following tables. These results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories, populations and locations.

LIAISON® XL Analyzer #1: Within-Instrument Precision

Sample ID	n	Mean IFN-γ (IU/mL)	Intra-run Lot 1 CV	Total within lot 1 CV	Intra-run Lot 2 CV	Total within lot 2 CV	Total across lots CV	Min value IFN-γ (IU/mL)	Max value IFN-γ (IU/mL)
P01	160	0.17	7.6%	12.1%	7.3%	12.8%	12.4%	0.120	0.234
P02	160	0.21	5.8%	11.6%	5.6%	9.4%	10.5%	0.156	0.257
P03	160	0.39	4.6%	10.8%	3.9%	10.3%	10.5%	0.294	0.484
P04	160	0.61	3.4%	9.8%	4.8%	9.0%	9.4%	0.479	0.745
P05	160	1.27	3.0%	9.8%	4.4%	9.6%	9.7%	0.961	1.52
P06	160	2.67	2.9%	7.7%	4.3%	7.6%	7.6%	2.16	3.04
P07	160	3.55	3.7%	8.7%	3.9%	7.3%	8.1%	2.92	4.14
P08	160	5.17	2.5%	7.1%	4.5%	7.6%	7.3%	4.36	6.08
P09	160	5.61	2.8%	7.4%	3.9%	8.1%	7.7%	3.93	6.72
P10	160	6.50	3.7%	7.7%	4.7%	7.9%	7.8%	5.27	7.58

LIAISON® XL Analyzer #2: Within-Instrument Precision

Sample ID	n	Mean IFN-γ (IU/mL)	Intra-run Lot 1 CV	Total within lot 1 CV	Intra-run Lot 2 CV	Total within lot 2 CV	Total across lots CV	Min value IFN-γ (IU/mL)	Max value IFN-γ (IU/mL)
P01	160	0.17	3.6%	10.7%	3.5%	9.1%	9.8%	0.120	0.222
P02	160	0.21	3.8%	10.1%	3.7%	8.3%	9.1%	0.153	0.255
P03	160	0.40	2.9%	9.2%	2.5%	8.2%	8.7%	0.312	0.497
P04	160	0.63	3.0%	9.1%	2.5%	8.8%	8.9%	0.506	0.768
P05	160	1.34	2.7%	9.3%	2.5%	10.3%	9.8%	1.05	1.65
P06	160	2.82	2.9%	9.5%	3.5%	9.5%	9.5%	2.31	3.44
P07	160	3.78	2.7%	10.3%	2.3%	9.9%	10.1%	3.08	4.49
P08	160	5.52	2.2%	9.7%	2.8%	10.2%	9.9%	4.52	6.63
P09	160	5.95	2.4%	9.3%	2.3%	10.7%	10.0%	4.93	7.24
P10	160	6.92	3.3%	10.2%	2.5%	10.9%	10.6%	5.69	8.36

15.3. Precision with LIAISON® XS Analyzer

A five-day precision study was conducted on three LIAISON® XS analyzers to verify the precision with the LIAISON® QuantiFERON®-TB Gold Plus Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol. Six (6) Li-Heparin samples containing spiked concentrations of native IFN-γ that span the assay range were used for the study.

The coded panel was tested on three LIAISON® XS analyzers, in six replicates in a single run per day, for 5 operative days. The mean IFN-γ level, the standard deviation, and the coefficient of variation (%CV) of the results were computed for each of the tested specimens, for each of the instruments, and across instruments.

Sample ID	n	Mean IFN-γ (IU/mL)	Intra-run CV%	Total within site CV%	Min value IFN-γ (IU/mL)	Max value IFN-γ (IU/mL)
PP-001	90	0.163	3.7%	4.4%	0.140	0.188
PP-002	90	0.373	3.0%	4.0%	0.332	0.417
PP-003	90	0.433	4.0%	4.6%	0.388	0.543
PP-004	90	0.853	3.3%	4.3%	0.732	0.980
PP-005	90	3.394	3.2%	4.4%	3.16	4.02
PP-006	90	7.672	3.2%	3.5%	6.80	8.25

15.4. High-dose hook effect

No high-dose hook effect was observed for IFN-γ concentrations up to 10,000 IU/mL.

16. CLINICAL PERFORMANCE

Agreement with a reference CE-mark assay was evaluated by assessing 435 subjects, of whom 337 subjects from a prospective population and 98 subjects from retrospective populations, collected from a TB diagnostic routine of 3 different European laboratories.

148 concordant positive results were observed in the population studied, on a total of 155 positive subjects with reference assay. In positive subjects, results in agreement were 95.48% (95% Confidence Interval: 90.92 – 98.17%).

262 concordant negative results were observed in the same population, on a total of 271 negative subjects with reference assay. In negative subjects, results in agreement were 96.68% (95% Confidence Interval: 93.80 – 98.47%).

9 subjects resulted Indeterminate with both assays.

The overall agreement (indeterminate subjects included) was 96.32% (95% Confidence Interval: 94.10 – 97.89%).

With regard to the LIAISON® XL concordant positive findings (148 patients), 3 subjects resulted classified as Positive by TB1 only, while 10 subjects were Positive by TB2 only. The other 135 specimens were reactive with both TB1 and TB2 tubes.

These results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories, populations and locations.

A Summary of safety and performance is available on EUDAMED.

For EU only: please be aware that any serious incident that has occurred in relation to this IVD medical device should be reported to DiaSorin Italia S.p.A. and to the Competent Authority of the EU Member State in which the user and/or the patient is established.

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