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| **QuantiFERON** |
| **Purpose** | This procedure provides instructions for performing QuantiFERON TB Gold Plus on the DiaSorin LIAISON XL®. |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing Quantiferon testing on the DiaSorin LIAISON XL®. |
| Principle | **Method:** Chemiluminescence immunoassay (CLIA)The LIAISON® QuantiFERON®-TB Gold Plus assay is an in vitro diagnostic test for the detection of interferon-y (IFN- y) 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- y 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 valuations 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. |
| **Clinical Significance** | 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 10.4 million new cases and 1.3 million deaths from TB in 2016 (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 symptomatic TB. Based on this definition, individuals with LTBI carry an increased risk of progression to active TB disease. However, an unknown but large number of those with LTBI will not develop active TB disease, either because their immune system persistently controls mycobacterial replication or because the mycobacteria are no longer viable.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 to cause 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 the risk of developing disease from latent infection (if present) is increased; 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 in contact with those with active TB, people living with human immunodeficiency virus [HIV] infection, or otherwise immunosuppressed persons because of medications or conditions such as uncontrolled diabetes). |
| **Instrument** | DiaSorin LIAISON® XLSunquest Method Code: **XL** |
| **Sunquest Test Code** | **QFTB**: QuantiFERON-TB, battery includes test for Nil, TB1, TB2, and Mitogen tubes.T1NIL: Nil tube IF-γ T2QT1: TB1 tube IF-γT3QT2: TB2 tube IF-γT4MT: Mitogen tube IF-γ |
| **Sample** | **Tube:** QFT-Plus blood Collection is set of 4 tubes in a Kit supplied by Qiagen.**Minimum volume**: 4 mL whole blood total, 0.8-1.2 mL per tube**Stability**: Before incubation: samples can sit at room temperature up to 16 hours.Post centrifuge: 28 days at 2-8C **Rejection criteria**: Unlabeled tube, over/under filled (must be at the black line), incorrect temperature storage conditions (see below)**Preparation:**For each patient, collect 4mL of blood and transfer 1 ml of blood directly into each of the QFT-Plus blood Collection Tubes. The black mark on the side of the tubes indicates the validated range of 0.8 to 1.2 ml. If the level of blood in any tube is outside of the indicator mark, reject the sample and obtain a new one.Immediately after filling the tubes, shake them ten (10) times just firmly enough to make sure the entire inner surface of the tube is coated with blood. This will dissolve antigens on the tube walls.Tube need to be Incubated at 37°C ± 1°C within 16 hours of collections. *Note:* Tubes need to be inverted 10x before being placed in the incubator.Incubate the QFT-Plus Blood Collection Tubes UPRIGHT at 37°C ± 1°C for 16 to 24 hours. The incubator does not require CO2 or humidification.After adequate incubation time, Centrifuge the tubes at 2000-3000g for 15 min.Place samples on the DiaSorin Liaison XL or store 2-4°C for 28 days. |

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| **Materials** | ***Product Description*** | ***Product Code*** | ***Stability*** |
|  | LIAISON QuantiFERON-TB Gold Plus (US) (200 tests)Reagent Kit contains:* Reagent Integral
* Conjugate ( white cap with red dot)
* Buffer R (green Cap)
* Calibrator A & B
 | MFR #311020CHC #34455 | Store at: 2-8°C. Unopened: Date on cartonOpened or on board: four (4) weeks stored at 2°-8°C or onboard the LIAISON® XL Analyzer. |
|  | LIAISON® Control QuantiFERON®-TB Gold Plus | MFR #311021CHC #34456 | Store at: 2-8° C Unopened: Date on vialOpened: 28 days at 2-8°C |
|  | QuantiFERON®-TB Gold Plus Collection kit (25 count) | MFR #622433CHC #34454 | Store at: RT until used or until lot expiration |
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| **Risk and Safety** | Refer to **safety data sheet on Children’s** [StarNet](https://starnet.childrenshc.org/applications/)Hazardous reagents are labeled and classified as follows: |
| Reagents: | [BUF|W], [BUF|R], [DIL] | [CAL|A] (lyophilized),[CAL|B] (lyophilized),[CONJ] (lyophilized) | [CAL|A] (reconstituted), [CAL|B] (reconstituted), [CONJ] (reconstituted) |
| Classification: | Skin sens. 1 H317 | Eye irrit. 2 H319Skin irrit. 2 H315Skin sens. 1 H317Aquatic Chronic 3 H412 | Skin sens. 1 H317 |
| Signal Word: | Warning | Warning | Warning |
| Symbols: |  |  |  |
| Hazard Statements: | H317 May cause an allergic skin reaction. | H315 Causes skin irritation.H317 May cause an allergic skin reaction.H319 Causes serious eye irritation.H412 Harmful to aquatic life with long lastingeffects. | H317 May cause an allergic skin reaction. |
| Precautionary statements: | P261 Avoid breathing dust/fume/gas/mist/vapours/spray.P280 Wear protective gloves/protective clothing/eye protection/face protection.P363 Wash contaminated clothing 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 easyto do. Continue rinsing.P273 Avoid release to the environment. | P261 Avoid breathing dust/fume/gas/mist/vapours/spray.P280 Wear protective gloves/protective clothing/eye protection/face protection.P363 Wash contaminated clothing before reuse. |
| Contains: | 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). | 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); gentamycin sulfate salt. | reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin® 300). |
|  | **Reagent containing sodium azide (Magnetic Particles [SORB])**Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to “Decontamination of Laboratory Sink Drains to Remove Azide Salts“, in the Manual |
| **Calibration** | Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each Calibration vial contains sufficient volume for the performance of at least 4 calibrations. Refer to the Operator's Manual or LIAISON XL® Quick Guide for calibration instructions.Recalibration is mandatory whenever at least one of the following conditions occurs:* A new lot of Reagent Integral or Starter Kit is used.
* The previous calibration was performed more than four (4) weeks prior.
* The LIAISON® XL Analyzer has been serviced.
* The values of the controls lie outside the expected ranges.

**Reconstitution of Calibrators:**Calibrators for LIAISON® QuantiFERON®-TB Gold Plus assay are supplied lyophilized.1. Reconstitute the vial contents with 2.0 mL of deionized or distilled water.
2. Allow the vials to stand for at least 15 minutes at 18°-25°C to achieve complete dissolution.
3. Mix vials thoroughly by gentle inversion; avoid foaming.
4. reconstituted Calibrators are stable for four (4) weeks when stored at 2°-8°C
5. place Vials on a T rack with barcodes facing out and place into the sample area of instrument
6. Go to the reagents tab on instrument and highlight QFT and press calibrate.

***Note****: 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 together in the same assay.* |
| **Analytical Measuring Range (AMR)** | Not Applicable |
| **Reagent Integral and conjugate Preparation**  | **How to prepare and load new integrals**1. Remove from refrigerated storage, maintaining upright orientation
2. Inspect Integral for leakage
3. Mix magnetic particle for 30 seconds
4. Seat test integral in Xcelerator for 30 seconds
5. Gently rotate the magnetic particle vial for 30 seconds
6. Remove new integral sealing flaps slowly
7. Remove all liquid from the surfaces of the membranes to prevent cross-contamination of the reagent vials by blotting with a kim wipe folded in half lengthwise
8. Open the reagent bay on the analyzer
9. Using a smooth motion, insert the integral into an unoccupied lane in the reagent area until it rests firmly against the docking pins at the rear. Let stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.

Note: if more than one integral of the same reagent is loaded, place the newest integral to the right of the old integral. The analyzer will sample from the left integral until empty, then move right.Conjugate preparation Conjugate for LIAISON® QuantiFERON®-TB Gold Plus assay is supplied lyophilized. Proper reconstitution of Conjugate is essential.1. Reconstitute the Conjugate vial contents with 4 mL of Buffer R (green top).
2. Seal the Conjugate vial with the stopper cap and mix thoroughly by gentle inversion 5 times. Avoid foaming.
3. Allow the Conjugate vial to stand at 18°-25°C for at least 15 minutes to achieve complete dissolution.
4. Conjugate solution must be loaded onto the LIAISON® XL Analyzer in the ancillary reagent area, immediately before use. Do not leave the reconstituted Conjugate at room temperature longer than the time required to process it on the Analyzer. Do not freeze.

*Note:* One Buffer R vial must be used to reconstitute one vial of lyophilized Conjugate, Discard the restStability: Reconstituted Conjugate is stable for 14 days when stored in capped vials at 2°-8°C |
| **Quality Control** | **Frequency:** Run 2 levels once per day of use. **Stability:** **Unopened:** Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date **Opened:** 4 weeks stored tightly capped at 2-8°C between uses. Mark vial with expiration date and initials upon opening.**Acceptable ranges:** * New lots of control should be verified that control values lie within the expected ranges provided on the certificate of analysis. A Bio-Rad Unity Real Time administrator (technical specialist or designee) should be notified several days before the new lot is used to allow for the new lot to be configured in Unity Real Time using the certificate of analysis.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Procedure** | Refer to the **Liaison XL Operating Procedure**.**LIAISON® XL Analyzer:** Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event that 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 for instruction. The LIAISON® XL Analyzer operational steps are as follows:1. Diluent and Magnetic Particles are dispensed into the LIAISON® XL Cuvette.2. Conjugate is dispensed into the LIAISON® XL Cuvette.3. Calibrators, Controls or samples are dispensed into the LIAISON® XL Cuvette.4. Incubation at 37°C.5. Wash is conducted using LIAISON® Wash/System Liquid.6. Assay Buffer W is dispensed.7. Incubation at 37°C.8. Wash is conducted using LIAISON® Wash/System Liquid.9. LIAISON® XL Analyzer Starter Kit reagents are added followed by measurement of emitted light.  |
| **Interpretation/****Results/Alert Values** | LIAISON® QuantiFERON®-TB Gold Plus assay results are interpreted using following algorithm (Table 1) that combines the results from each of the four QFT-Plus Blood Collection Tubes. The result (i.e., amount of analyte IFN-γ) for each blood collection tube is reported in International Units per mL (IU/mL). Although the assay detects IFN- γ quantitatively, the interpretation of the result for a single patient is strictly qualitative. The magnitude of the amount of measured IFN- γ cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. ***Note:*** *Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting LIAISON QuantiFERON-TB Gold Plus assay results. See general guidance on the diagnosis and treatment of TB disease and LTBI*[*https://www.cdc.gov/tb/publications/guidelines/default.htm*](https://www.cdc.gov/tb/publications/guidelines/default.htm)*.* Responses to the Mitogen positive control and occasionally to TB antigen can be above the assay range. Cases of undetectable response might be observed. This has no impact on test result. 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**

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| **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% Nil | <0.35 OR >0.35 and <25% Nil | >0.5 | Negative | *M. tuberculosis* infection NOT likely |
| <0.35 OR >0.35 and <25% Nil | <0.35 OR >0.35 and <25% Nil | <0.5 | Indeterminate\*\* | Likelihood of *M. tuberculosis* infection cannot be determined |
| >8.0 | Any |

\*Where M. tuberculosis infection is not suspected, initially positive results should be confirmed by re-testing 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.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. |

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| **Dilutions** | Do not dilute. See result Reporting. |
| **Reference Intervals** | **Reference value: Negative**; *M. tuberculosis* infection NOT likely**Abnormal values:** **Positive**; *M. tuberculosis* infection likely**Indeterminate**; Likelihood of *M. tuberculosis* infection cannot be determined |
| Limitations | 1. Grossly hemolyzed, icteric or lipemic samples, as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested.
2. The performance characteristics of the test in the following groups of individuals has not been extensively evaluated: individuals younger than age 18 years, pregnant women, individuals with impaired or altered immune functions or other clinical conditions (e.g. HIV infection, transplant recipients, hematological disorders, malignancies, diabetes, chronic Renal failure).
3. To obtain an accurate result interpretation for a patient, combine only the results from tubes collected from the patient in the same sampling session.
4. Inaccurate or indeterminate results may occur if strict adherence to the LIAISON® QuantiFERON®-TB Gold Plus assay and QuantiFERON®-TB Gold Plus Blood Collection Tube instructions is not exercised.
5. Bacterial contamination or heat inactivation of the specimens may affect the test results.
6. The four individual blood collection tube results of a patient sample can be combined to determine the final qualitative interpretation only if assay testing of subsequent tube(s) occurs within ≤18 hours of testing of the initial tube, and all tubes are maintained at 2°-8°C prior to testing.
7. In order to ensure correct correlation of the result of each assay tube and the interpretation, a conversion factor must not be set on the LIAISON® XL Analyzer.
8. 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.
9. 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.
	1. 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.
	2. 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 testing should be considered.
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| **Result Reporting** | Results in Sunquest following LIS procedures for OEM. Comments are automatically appended when resulting in OEM or MEM using the LIAS worksheet. Sunquest will automatically determine one of the following Result/Interpretation based upon the numerical results from each tube and the algorithm stated in Table 1.* **Indeterminate**. The comment “Likelihood of *M. tuberculosis* infection cannot be determined” is appended.
* **Positive**. Test must be repeated in duplicate prior to reporting as positive. The comment “*M*. *tuberculosis* infection likely” is appended.
* **Negative**. The comment “*M. tuberculosis* infection NOT likely” is appended.
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| **Alternate Methods** | * When test performance does not meet quality standards, consult the technical specialist or Medical Director, and refer testing to Mayo Medical Laboratory.
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| **References** | 1. DiaSorin QuantiFERON Instructions for Use, LIAISON® QuantiFERON®-TB Gold Plus ([REF] 311020) 1 / 20 EN - 200/007-002, 01 - 2019-11
2. DiaSorin QuantiFERON Controls Instructions for Use, LIAISON® Control QuantiFERON®-TB Gold Plus ([REF] 311021), 18 / 20 EN - 200/007-002, 01 - 2019-11
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| **Appendices** |  |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Stephen Gripentrog/Matt Johnson | 8/29/2022 | Initial Version |
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