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Changes: §1, §4.1, §10.1;
Deletions: §6;

LIAISON® LymeDetect® (REF 311030)

1. INTENDED PURPOSE

The LIAISON® LymeDetect® is a qualitative assay based on three combined chemiluminescent immunoassays (CLIA) for the early diagnosis of Lyme Borreliosis caused by *Borrelia burgdorferi sensu lato* (including strains of *B. burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*) in humans in conjunction with clinical findings. The combined result of the three assays is intended as an aid in the diagnosis of early *Borrelia burgdorferi sensu lato* infection, in subjects with clinical evidences or suspected tick bite, or whenever a Borrelia infection may be suspected.

The interpretation of the results is done through an algorithm combining the following three assays included in the pack:

– the **LIAISON® LymeDetect® IgG (REF 311032)**: an indirect test for the *in vitro* qualitative determination of specific IgG antibodies to *Borrelia burgdorferi sensu lato* (including strains *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, *Borrelia afzelii*) in human serum and Li-Hep plasma specimens.
– the **LIAISON® LymeDetect® IgM (REF 311033)**: an indirect test for the *in vitro* qualitative determination of specific IgM antibodies to *Borrelia burgdorferi sensu lato* (including strains *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, *Borrelia afzelii*) in human serum and Li-Hep plasma specimens.
– the **LIAISON® QuantiFERON® LymeDetect® (REF 311034)**: a direct test for the detection of IFN-γ in human lithium heparin plasma specimens. The immunoassay can identify *in vitro* responses to a peptide antigens cocktail associated with *Borrelia burgdorferi sensu lato* infection, that stimulates cells in heparinized whole blood collected with the LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes. Although the assay quantitatively detects the IFN-γ, the interpretation of the result for a single patient is strictly qualitative.

The tests must be performed on the LIAISON® XL and LIAISON® XS analyzers only.

2. SUMMARY AND EXPLANATION OF THE TEST

Lyme disease or Borreliosis is a tick-borne infectious disease caused by spirochetes of the *Borrelia burgdorferi sensu lato* complex, mainly *B. Burgdorferi sensu stricto*, *B. afzelii* and *B. garinii* [1,2].

The first manifestation of the disease could be a localized rash known as erythema migrans (EM) which in most cases may resolve spontaneously. However, if left untreated, disseminated Lyme borreliosis can develop, manifesting in more severe forms such as neuroborreliosis or Lyme arthritis [3-5].

Lyme disease is treated with antimicrobials with activity against *Borrelia burgdorferi*. The goals of treatment are the eventual resolution of signs and symptoms of infection, with prevention of relapsed active infection or new complications of infection. The appropriate antibiotic and duration of the treatment is based on the signs and symptoms of disease manifestation and on patient status [6].

The diagnosis of Lyme disease should be made clinically and in conjunction with laboratory serology tests.

In the presence of EM, international guidelines recommend a clinical diagnosis with no serological assays to be performed [6,7]. However, EM are not always present, are frequently atypical and in approximately 50% of cases there is no known history of a tick bite, which makes diagnosis considerably more difficult [8].

Conventional serological testing for Lyme disease uses a two-tier solution employing an enzyme immunoassay (EIA) or chemiluminescence immunoassay (CLIA) in the first tier followed by confirmatory IgM/IgG immunoblotting in the second tier for positive or equivocal results. Diagnostic performance is often unsatisfactory, especially in early infection, where standard two-tier testing (sTTT) rarely exceeds sensitivities of 50% [9,10].

Branda et al. [11] discussed an alternative serology test strategy named modified two-tier testing (mTTT). The mTTT algorithm includes an EIA plus another confirmatory EIA for patients testing positive or equivocal. However, even with this alternative antibody detection assay sequence for patients suspected with an early infection, sensitivity ranged from only 36% to 54% [11].

T-lymphocyte-mediated responses to *Borrelia burgdorferi* antigens result in the specific release of cytokines, especially interferon-gamma, which could be measured using interferon gamma release assays (IGRAs) [12].

Branda et al. [12] highlighted that: “In some cases, strong interferon-gamma responses can be observed shortly after initial infection, and it is possible that IGRAs could be capable of detecting the infection earlier than antibody tests during the serological window period”.

Callister et al. [9] supported the necessity to have a test that monitors T-cell activation, because it might be a useful adjunct to traditional serological testing methods, especially because the results may provide more accurate information on the presence of active infection compared to antibody responses.

A study conducted by Callister et al. [9] demonstrated that combining information from standard serological testing with IGRA results led to higher sensitivity for early Lyme Disease detection (83% combined IGRA serology vs 59% serology only).

3. PRINCIPLE OF THE PROCEDURE

The three methods included in the pack are:

3.1. LIAISON® LymeDetect® IgG (REF 311032)

The method for qualitative determination of specific IgG to *Borrelia burgdorferi* is an indirect chemiluminescence immunoassay (CLIA). Recombinant antigens specific for *Borrelia burgdorferi* are used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate).

During the first incubation, *Borrelia burgdorferi* antibodies present in calibrators, samples or controls bind to the solid phase.

During the second incubation, the antibody conjugate reacts with IgG to *Borrelia burgdorferi* already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence 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 IgG to *Borrelia burgdorferi* concentration present in calibrators, samples or controls.

3.2. LIAISON® LymeDetect® IgM (REF 311033)

The method for qualitative determination of specific IgM to *Borrelia burgdorferi* is an indirect chemiluminescence immunoassay (CLIA). Recombinant antigens are used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, *Borrelia burgdorferi* antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with IgM to *Borrelia burgdorferi* already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence 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 IgM to *Borrelia burgdorferi* concentration present in calibrators, samples or controls.

3.3. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

The method used to determine IFN-γ is a direct sandwich chemiluminescence immunoassay (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 chemiluminescence 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 IFN-γ concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

Three (3) Integrals of reagents are provided in the pack, one for each of the assays to be combined.

4.1. LIAISON® LymeDetect® IgG (REF 311032)

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with *Borrelia burgdorferi* VIsE (*Borrelia garinii* strain pBi) recombinant antigen (obtained in *E. coli*) (approx. 100 µg/mL), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (3.8 mL)	CAL 1	Human serum/plasma containing low *Borrelia burgdorferi* IgG levels (approx. 15 AU/mL), BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (3.8 mL)	CAL 2	Human serum/plasma containing high *Borrelia burgdorferi* IgG levels (approx. 175 AU/mL), BSA, phosphate buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Specimen diluent (28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (23 mL)	CONJ	Mouse monoclonal antibodies to human IgG (minimum 10 ng/mL) conjugated to an isoluminol derivative, mouse monoclonal antibodies to human IgG, BSA, PBS buffer, 0.2% ProClin® 300, preservatives.
Number of tests		65

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the reagent integral.

4.2. LIAISON® LymeDetect® IgM (REF 311033)

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with OspC (*Borrelia afzelii* strain pKo) (approx. 100 µg/mL) and VIsE (*Borrelia garinii* strain pBi and *Borrelia sensu stricto* strain B31) (approx. 50 µg/mL) recombinant antigens (obtained in *E. coli*), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (0.9 mL)	CAL 1	Human serum/plasma containing low *Borrelia burgdorferi* IgM levels (approx. 17.5 AU/mL), BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations are referenced to an in-house antibody preparation.
Calibrator 2 (0.9 mL)	CAL 2	Human serum/plasma containing high *Borrelia burgdorferi* IgM levels (approx. 125 AU/mL), BSA, phosphate buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations are referenced to an in-house antibody preparation.
Specimen diluent (28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (23 mL)	CONJ	Mouse monoclonal antibodies to human IgM (minimum 10 ng/mL) conjugated to an isoluminol derivative, BSA, phosphate buffer, 0.2% ProClin® 300, preservatives.
Number of tests		65

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the reagent integral.

4.3. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

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.
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.
Buffer R (2 x 4.5 mL)	BUF R	Streptavidin conjugated with isoluminol derivative (approx. 4.5 µg/mL), BSA, casein, phosphate buffer, 0.2% ProClin® 300, gentamicin sulfate 0.1 g/L, non-specific IgG (mouse polyclonal), detergents.
Conjugate (lyophilized, 2 x 4 mL)	CONJ	Biotinylated antibody to human IFN-γ (mouse monoclonal) (approx. 15 µg /mL), HEPES buffer, BSA, casein, non-specific IgG (mouse polyclonal), 0.2% ProClin® 300, gentamicin sulfate 0.1 g/L, detergents.
2 labels for reconstituted Conjugate.		
1 Barcoded label for reconstituted Calibrator A.		
1 Barcoded label for reconstituted Calibrator B.		

Buffer R, Diluent, Assay Buffer W and Magnetic particles are provided ready-to-use. Calibrators and Conjugates 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 Kit (REF 310996).

Additionally required materials

LIAISON® Control LymeDetect® (REF 311031).
LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes (tubes for hematic withdrawal) (REF 311035).

Additionally available tool

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 leaking 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. However, as no test method can offer absolute assurance of the absence of pathogens, 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 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).

For correct combination of dosage, do not use integrals from different lots of LIAISON® LymeDetect® kit (311030). The correct combination of lots to be used is indicated on the label of each integral.

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:

LIAISON® LymeDetect® IgG (REF 311032)

REAGENTS:	CAL 1, CAL 2, CONJ, DIL SPE
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 labeled as EUH210 safety data sheets available on request.
For additional information see Safety Data Sheets available on www.diasorin.com

LIAISON® LymeDetect® IgM (REF 311033)

REAGENTS:	CAL 1, CAL 2, DIL SPE, CONJ
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 labeled as EUH210 safety data sheets available on request.
For additional information see Safety Data Sheets available on www.diasorin.com

LIAISON® QuantiFERON® LymeDetect® (REF 311034)

REAGENTS:	BUF W, BUF R, DIL	CAL A (lyophilized), CAL B (lyophilized), CONJ (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 + 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).	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, CONJ are classified as indicated below:

REAGENTS:	CAL A (reconstituted), CAL B (reconstituted), CONJ (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 labeled 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

7.1. COMMON PREPARATIONS for LIAISON® LymeDetect® IgG, LIAISON® LymeDetect® IgM and LIAISON® QuantiFERON® LymeDetect®

7.1.1. REAGENT INTEGRAL

The following information is to be considered applicable for all the three integrals available in the pack.

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 at the magnetic particle compartment until the colour 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

Warning – Only for the kit LIAISON® LymeDetect® IgM before removing the seals from the vials and before each calibration, gently shake the reagent integral, avoiding foam formation.

– 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.

The following information is to be considered specific for the different integrals available in the pack.

7.2. SPECIFIC PREPARATIONS for LIAISON® QuantiFERON® LymeDetect® (REF 311034)

7.2.1. CONJUGATE AND BUFFER R

Conjugate for LIAISON® QuantiFERON® LymeDetect® assay is supplied lyophilized. Buffer R is provided in liquid form. Conjugate and Buffer R 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 Conjugate is automatically checked by the analyzer. Each vial of the Conjugate reagent allows 200 tests to be performed.

Each Buffer R vial must be used to reconstitute one vial of lyophilized Conjugate.

Do not pool the contents of different Buffer R vials, even if they belong to the same lot. Discard the remaining volume of a vial of Buffer R after using it for Conjugate reconstitution.

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

Proper reconstitution of Conjugate is essential.

– Reconstitute the Conjugate vial contents with 4 mL of Buffer R.
– Mix the Conjugate vial thoroughly by gentle inversion 5 times after sealing with a stopper and cap. Avoid foaming.
– Allow the Conjugate vial to stand for at least 15 minutes at 18-25°C to achieve complete dissolution.
– Affix the appropriate, additionally provided label to the Conjugate vial.
– Once reconstituted, refer to paragraph 8 to store the Conjugate.
– The reconstituted Conjugate solution must be loaded onto the instrument in the ancillary reagent area immediately before use.
– For details on the reagent use in the ancillary reagent area on board the instrument, refer to the analyzer operator's manual.

The original vial label refers only to the lyophilized Conjugate. Once reconstituted, pursuant to EC Regulation 1272/2008 (CLP), the Conjugate is classified as Skin sens. 1A H317 and Aquatic chronic 3 H412. For more details, refer to paragraph 6.

7.2.2. CALIBRATORS

Calibrators for LIAISON® QuantiFERON® LymeDetect® 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.

– 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 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 section of LIAISON® Control LymeDetect® for proper preparation and handling instructions.

8. REAGENT STORAGE AND STABILITY

8.1. COMMON PREPARATIONS for LIAISON® LymeDetect® IgG, LIAISON® LymeDetect® IgM and LIAISON® QuantiFERON® LymeDetect®

8.1.1. REAGENT INTEGRAL

The following information is to be considered applicable for all three integrals available in the pack.

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.

The following information is to be considered specific for the different integrals available in the pack.

8.2. SPECIFIC PREPARATIONS for LIAISON® QuantiFERON® LymeDetect® (REF 311034)

8.2.1. CONJUGATE

– **Lyophilized**: stable at 2-8°C until the expiry date. Upon receipt, the Conjugate must be stored at 2-8°C in an upright position to prevent adherence of the lyophilizate to the vial cap.
– **Reconstituted**: stable for 14 days when properly stored at 2-8°C between two successive uses, in the respective capped vials.

After reconstitution, the conjugate must be stored in an upright position to prevent adherence of the solution to the vial cap.

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

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

8.2.2. BUFFER R

– Stable at 2-8°C until the expiry date. The Buffer R must be stored in an upright position to prevent adherence of the solution to the vial cap.
– Once open, the Buffer R vial must be used immediately to reconstitute the lyophilized Conjugate.

Do not leave the Buffer R at room temperature longer than the time required to process it. Do not freeze.

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

8.2.3. 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

9.1.1. LIAISON® LymeDetect® IgG (REF 311032) and LIAISON® LymeDetect® IgM (REF 311033)

The correct specimen type must be used in the assay. Following matrices have been tested and may be used:

– serum;
– lithium-heparin plasma;
– EDTA plasma;
– Citrate plasma.

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.

Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.

Uncontrolled transport conditions (in terms of temperature and time) may cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore, not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010, [13]).

A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells or gel separator. The following storage conditions showed no significant differences:

– room temperature storage should be avoided;
– 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– up to 4 freeze-thaw cycles, however multiple freeze-thaw cycles should be avoided.

If samples are stored frozen, mix thawed samples well before testing.

Further centrifugation of specimens removed from red cells, clot or gel separator (suggested between 3,000 and 10,000 g for 10 minutes) is recommended in order to guarantee the consistency of results whenever one of the following conditions is identified:

– Samples previously centrifuged and stored at 2°-8°C;
– Samples with particulate matter, fibrin, turbidity, lipaemia or erythrocyte debris;
– Samples frozen and thawed;
– Samples requiring repeat testing.

Specimens with a lipid layer on the top should be transferred into a secondary tube, taking care to transfer only the clarified material.

Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Heat inactivation of the specimens may affect the test results. Check for and remove air bubbles before assaying.

The minimum volume required for a single determination using one single tube is 200 µL of specimen (20 µL specimen for LIAISON® LymeDetect® IgG + 30 µL specimen for LIAISON® LymeDetect® IgM + 150 µL dead volume). No further manipulation is required because the instrument automatically dilutes specimens before testing.

9.1.2. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

LIAISON® QuantiFERON® LymeDetect® is validated only with whole blood specimens collected, handled and processed with LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes.

Whole blood must be collected referring to the LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes instructions for use, 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 haemolysis may appear. Haemoglobin 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 LIAISON® QuantiFERON® LymeDetect® 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 LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes, refer to the instructions for use of the tubes. Specimens harvested in secondary tubes can be tested within 28 days if stored at 2-8°C, or after an extended period 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.

10.1. LIAISON® LymeDetect® IgG (REF 311032) and LIAISON® LymeDetect® IgM (REF 311033)

Each calibration solution allows eight (8) calibrations to be performed.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
- A new lot of Starter Kit is used.
- The previous calibration was performed more than four (4) weeks before.
- Each time a new lot of integral is used.
- Control values lie outside the expected ranges.
- **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 local DiaSorin technical support or representative.

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

Warning – Only for the kit LIAISON® LymeDetect® IgM before removing the seals from the vials and before each calibration, gently shake the reagent integral, avoiding foam formation.

10.2. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

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 printed also on the Reagent Integral Label.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
- A new Starter Kit lot is used.
- The previous calibration was performed more than four (4) weeks before.
- A new Reagent Integral lot is used.
- **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.

11.1. LIAISON® LymeDetect® IgG (REF 311032)

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

11.2. LIAISON® LymeDetect® IgM (REF 311033)

1. Dilute specimens with Specimen diluent.
2. Dispense Specimen diluent.
3. Dispense coated magnetic particles.
4. Dispense calibrators, controls or diluted specimens into the reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. Dispense conjugate into the reaction module.
8. Incubate.
9. Wash with Wash/System liquid.
10. Add the Starter Kit and measure the light emitted.

11.3. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

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 LymeDetect® (REF 311031):

(a) At least once per day of use, before running the test,

(b) Whenever the kit is calibrated,

(c) Whenever a new lot of Starter Reagents is used,

(d) 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

Each subject must be tested with the 3 integrals included in the pack. Each of the 3 assays generates a result and an interpretation of the result for the single marker. The classifications obtained from the individual markers must then be combined through the final algorithm.

13.1. LIAISON® LymeDetect® IgG (REF 311032)

The analyzer automatically calculates *Borrelia burgdorferi* IgG antibody concentrations expressed as arbitrary units (AU/mL) and grades the results. For details, refer to the analyzer operator's manual.

Calibrators and controls may give different RLU or dose results on LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 5 to 240 AU/mL *Borrelia burgdorferi* IgG.

The threshold value that distinguishes between the presence and absence of anti-*Borrelia burgdorferi* IgG corresponds to 15 AU/mL.

Sample results should be interpreted as follows:

Samples with *Borrelia burgdorferi* IgG concentrations below 15 AU/mL should be graded *negative*.

Samples with *Borrelia burgdorferi* IgG concentrations equal to or above 15 AU/mL should be graded *positive*.

13.2. LIAISON® LymeDetect® IgM (REF 311033)

The analyzer automatically calculates *Borrelia burgdorferi* IgM antibody concentrations expressed as arbitrary units (AU/mL) and grades the results. For details, refer to the analyzer operator's manual.

Calibrators and controls may give different RLU or dose results on LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 2 to 190 AU/mL *Borrelia burgdorferi* IgM.

The threshold value that distinguishes between the presence and absence of anti-*Borrelia burgdorferi* IgM corresponds to 40 AU/mL.

Sample results should be interpreted as follows:

Samples with *Borrelia burgdorferi* IgM concentrations below 40 AU/mL should be graded *negative*.

Samples with *Borrelia burgdorferi* IgM concentrations equal to or above 40 AU/mL should be graded *positive*.

13.3. Interpretation of results of LIAISON® QuantiFERON® LymeDetect® (REF 311034)

LIAISON® QuantiFERON® LymeDetect® results are interpreted using the following criteria. 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 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 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>
<tr><th>Nil (IU/mL)</th><th>LYME minus Nil (IU/mL)</th><th>Mitogen minus Nil (IU/mL)</th><th>Results for LIAISON® QuantiFERON® LymeDetect®</th></tr>
<tr><td rowspan="3">≤ 8.0</td><td>≥ 0.20 and
≥ 25% of Nil</td><td>Any</td><td>Positive</td></tr>
<tr><td rowspan="2">< 0.20
OR
≥ 0.20 and
< 25% of Nil</td><td>≥ 0.5</td><td>Negative</td></tr>
<tr><td>< 0.5</td><td rowspan="2">Indeterminate</td></tr>
<tr><td>> 8.0</td><td colspan="2">Any</td></tr>
</table>

13.4. FINAL result interpretation for LIAISON® LymeDetect® (REF 311030)

Results indicated by ASYc optional SW	NEG	POS_A(*)	POS_B(*)	POS_C(*)	POS_D(*)	POS_E	POS_F	POS_G	POS_H	POS_I	POS_J	IND
QuantiFERON LymeDetect	NEG	POS(*)	POS(*)	POS(*)	POS(*)	NEG	NEG	NEG	IND	IND	IND	IND
LymeDetect IgM	NEG	NEG	POS	POS	NEG	POS	POS	NEG	POS	NEG	POS	NEG
LymeDetect IgG	NEG	NEG	NEG	POS	POS	POS	NEG	POS	NEG	POS	POS	NEG
Result/ Interpretation	Negative. Lyme Borreliosis NOT likely	Positive. Suspected EARLY Lyme Borreliosis (only in conjunction with clinical findings - Skin and/or Systemic symptoms)	Positive. EARLY Lyme Borreliosis Likely	Positive. EARLY Lyme Borreliosis Likely	Positive. EARLY Lyme Borreliosis Likely	Positive. EARLY Lyme Borreliosis Likely	Positive. Suspected EARLY Lyme Borreliosis	Positive. Suspected Lyme Borreliosis AT ANY STAGE	Positive. Suspected EARLY Lyme Borreliosis	Positive. Suspected Lyme Borreliosis AT ANY STAGE	Positive. EARLY Lyme Borreliosis Likely	Indeterminate. Likelihood of EARLY Lyme Borreliosis cannot be determined

(*) In the unlikely event that a subject's result is reported as positive and their Mitogen minus Nil result is less than 0.5 IU/mL, there is a possibility of a false positive result due to an exchange between the LYME antigen and Mitogen samples. Before recording this positive result, make sure that the LYME antigen and Mitogen samples have not been exchanged.

The calculations of the intermediate results and the final interpretation of the results of the LIAISON® LymeDetect® test can also be performed using the optional LIAISON® ASYc software. In the case mentioned above, the ASYc software uses the symbol "*" to underline that the result is due to a possible exchange of samples.

For more information, contact your local DiaSorin representative.

To obtain the correct correspondence of results between the tubes and their interpretation, it is recommended not to set any conversion factor on the analyzer.

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 only if assayed with the same lot** and with the same type of instrument LIAISON® XL Analyzer or LIAISON® XS Analyzer.
3. **LIAISON® QuantiFERON® LymeDetect®**: three 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 within the maximum sample stability.
4. **LIAISON® LymeDetect®**: results of the same patient can be combined for the final qualitative interpretation only if the last result is obtained within forty-two (42) hours from the first result and within the maximum sample stability.
5. Test results are reported in IU/mL for IFN-γ detection and in AU/mL for detection of IgG and IgM, but the interpretation of the patient is strictly qualitative.
6. A negative result does not preclude the possibility of *B. burgdorferi* infection or Lyme disease: false-negative results can be due to the stage of infection, 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.
7. A positive result should not be considered the only or definitive basis for determining infection with *B. burgdorferi*. Incorrect performance of the assay may cause false-positive responses.
8. Test results are qualitatively reported as positive, negative, or indeterminate. However, the diagnosis of an infectious disease must be evaluated in conjunction with other clinical findings, diagnostic procedures, and the physician's judgment.
9. Combined LymeDetect® results classified as indeterminate must be confirmed by repeating the LymeDetect® test on residual specimens or other aliquots from the same patient (if available) - within the stability limits thereof - and evaluated in combination with the clinical observation and risk assessment.
10. A skillful technique and strict adherence to the instructions of the LIAISON® QuantiFERON® LymeDetect® Blood Collection Tubes and of the immunoassay are necessary in order to obtain reliable results.
11. Proper reconstitution of the Conjugate is essential.
12. Grossly haemolyzed, icteric or lipaemic 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. Antibiotic therapy during the early stages of the disease often prevents development of antibody response.
15. 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.
16. The presence of rheumatoid factor and infectious mononucleosis must be excluded in patients with an isolated positive result for *Borrelia burgdorferi* IgM. Polyclonal stimulation of B lymphocytes during infectious mononucleosis, in fact, may result in non-specific induction of synthesis of *Borrelia burgdorferi* antibodies, especially of the IgM class.
17. Integrals may not be exchanged between analyzer types (LIAISON® XL and LIAISON® XS). Once an integral has been loaded on a specific analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups must not be concluded between analyzer types. These must be accomplished on one particular analyzer type (LIAISON® XL or LIAISON® XS).
18. The individual results obtained with LIAISON® LymeDetect IgG®, LIAISON® LymeDetect® IgM and LIAISON® QuantiFERON® LymeDetect® are not intended for any diagnosis based on the specific marker. According to the Intended Use of LIAISON® LymeDetect®, the qualitative result interpretation can only be performed by combining the individual assay results through the validated Algorithm reported in paragraph 13. INTERPRETATION OF RESULTS.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. LIAISON® LymeDetect® IgG (REF 311032)

15.1.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance in serum or plasma samples was not affected by anticoagulants (sodium citrate, EDTA, heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 40 mg/dL bilirubin), cholesterol (up to 500 mg/dL), human anti-mouse antibodies (HAMA; up to 234.9 ng/mL), rheumatoid factor (anti-Fc immunoglobulin; up to 333 IU/mL) or by freeze-thaw cycles of samples.

Cross-reactions. As a rule, the presence of potentially cross-reactive antibodies in serum or plasma samples does not interfere in the assay. The antibodies investigated were: (a) immunoglobulins to various infectious agents – such as EBV, *Treponema pallidum* or *Toxoplasma gondii* – (b) anti-nuclear (ANA) antibodies. The following table summarizes the study performed.

Clinical condition	Number of cases	IgG positive result
Past EBV infection	17	1
Syphilis	48	3
Past toxoplasmosis	14	0
Anti-nuclear antibodies	20	0
Total number of specimens tested	99	4

15.1.2. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

Repeatability. Twenty replicates were performed in the same run to evaluate repeatability.

Repeatability	1	2	3	4	5	6	7	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (AU/mL)	7.253	22.86	24.88	42.35	52.98	88.03	106.4	31.70
Standard deviation	0.37	1.51	2.34	2.98	2.58	8.69	10.90	1.12
Coefficient of variation (%)	5.0	6.6	9.4	7.0	4.9	9.9	10.2	3.5
Min. value	6.280	19.76	17.79	35.74	48.55	59.36	80.08	29.53
Max. value	7.844	25.45	27.33	47.12	59.04	96.45	118.0	33.13

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) to evaluate reproducibility.

Reproducibility	1	3	8	4	5	6	7	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (AU/mL)	7.416	28.31	29.24	47.24	61.72	94.58	116.3	34.66
Standard deviation	0.41	2.49	1.97	3.00	5.10	11.70	15.35	2.57
Coefficient of variation (%)	5.5	8.8	6.8	6.4	8.3	12.4	13.2	7.4
Min. value	6.592	23.99	25.90	41.44	53.59	69.30	77.96	30.84
Max. value	7.959	32.79	34.52	52.23	70.10	125.3	140.5	39.61

Lot-to-Lot Reproducibility. Samples tested in singleton on five different LIAISON® XL instruments on four different batches.

Lot-to-Lot Reproducibility	A	B	C	D	Positive control	*Negative control
Mean (AU/mL/RLUs)	15.285	8.649	164	74.13	35.945	1658
Inter-lot coefficient of variation (%)	11.5	8.5	2.0	7.8	1.0	0.0

* Data calculated on RLUs value due to doses out of the assay range.

15.1.3. Precision with LIAISON® XS Analyzer

A five-day precision study was conducted on three LIAISON® XS analyzers to verify the precision. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 6 frozen samples was used for the study.

The samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels.

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 value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability.

Repeatability	9	10	11	12	13	14	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (AU/mL)	6.913	9.812	16.70	29.40	39.94	85.14	29.52
Standard deviation	0.161	0.217	0.517	0.680	0.932	2.657	0.746
Coefficient of variation (%)	2.3	2.2	3.1	2.3	2.3	3.1	2.5
Min. value	6.038	8.913	12.89	26.52	36.30	74.50	25.58
Max. value	7.668	10.80	17.64	31.02	43.34	92.79	32.14

Reproducibility.

Reproducibility	9	10	11	12	13	14	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (AU/mL)	6.913	9.812	16.70	29.40	39.94	85.14	29.52
Standard deviation	0.190	0.263	0.593	0.772	1.104	3.009	1.001
Coefficient of variation (%)	2.7	2.7	3.6	2.6	2.8	3.5	3.4
Min. value	6.038	8.913	12.89	26.52	36.30	74.50	25.58
Max. value	7.668	10.80	17.64	31.02	43.34	92.79	32.14

15.1.4. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than actually present. However, a well-optimized two-step method excludes grossly underestimated results because the analytical signals remain consistently high (saturation curve).

Analysis of saturation effect was evaluated by testing three high-titred serum samples positive for *Borrelia burgdorferi* IgG.

All samples resulted in estimated concentration values above the assay range that would be expected with high-titred samples, indicating no sample misclassification.

15.2. LIAISON® LymeDetect® IgM (REF 311033)

15.2.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance in serum or plasma samples was not affected by anticoagulants (sodium citrate, EDTA, heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 40 mg/dL bilirubin), cholesterol (up to 500 mg/dL), human anti-mouse antibodies (HAMA; up to 234.9 ng/mL), rheumatoid factor (anti-Fc immunoglobulin; up to 333 IU/mL), or by freeze-thaw cycles of samples.

Cross-reactions. As a rule, the presence of potentially cross-reactive antibodies in serum or plasma samples does not interfere in the assay. The antibodies investigated were: (a) immunoglobulins to various infectious agents – such as EBV, *Treponema pallidum* or *Toxoplasma gondii* – (b) anti-nuclear (ANA) antibodies and rheumatoid factor (anti-Fc immunoglobulin) antibodies. The following table summarizes the studies performed.

Clinical condition	Number of cases	IgM positive result
Acute primary EBV infection	10	0
Syphilis	5	0
Acute primary toxoplasmosis	14	0
Anti-nuclear antibodies	16	0
Rheumatoid factor	10	0
Total number of specimens tested	55	0

15.2.2. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

Repeatability. Twenty replicates were performed in the same run to evaluate repeatability.

Repeatability	1	2	3	4	5	6	7	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (AU/mL)	11.80	28.82	35.31	44.90	49.61	67.18	112.8	61.39
Standard deviation	0.34	0.86	0.84	1.14	1.06	1.80	3.25	2.69
Coefficient of variation (%)	2.9	3.0	2.4	2.6	2.1	2.7	2.9	4.4
Min. value	11.08	27.16	33.45	43.17	47.33	64.17	108.6	56.64
Max. value	12.45	30.23	36.84	47.34	51.58	71.02	121.1	67.32

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) to evaluate reproducibility.

Reproducibility	1	8	2	9	4	5	6	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (AU/mL)	13.11	18.24	29.55	31.17	45.20	57.52	72.18	63.12
Standard deviation	1.20	1.39	2.60	2.36	3.44	11.55	7.52	4.50
Coefficient of variation (%)	9.2	7.6	8.8	7.6	7.6	20.1	10.4	7.1
Min. value	10.89	14.90	24.83	25.54	37.12	43.48	58.62	53.25
Max. value	14.99	21.69	33.15	33.67	50.81	80.99	89.51	69.59

Lot-to-Lot Reproducibility. Samples tested in singleton on five different LIAISON® XL instruments on four different batches.

Lot-to-Lot Reproducibility	A	B	C	D	E	F	Positive control	Negative control
Mean (AU/mL)	5.973	10.74	22.77	47.14	71.35	145.1	58.35	2.921
Inter-lot coefficient of variation (%)	8.5	4.6	4.0	2.0	2.5	9.8	4.7	13.9

15.2.3. Precision with LIAISON® XS Analyzer

A five-day precision study was conducted on three LIAISON® XS analyzers to verify the precision. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 6 frozen samples containing different concentration of analyte and kit controls was 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 dose mean, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same test to evaluate repeatability. 6 samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Repeatability	10	11	12	13	14	15	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (AU/mL)	7.151	15.87	24.16	83.39	32.17	41.74	58.01
Standard deviation	0.127	0.285	0.451	1.859	0.653	0.846	1.030
Coefficient of variation (%)	1.8	1.8	1.9	2.2	2.0	2.0	1.8
Min. value (AU/mL)	6.652	14.49	22.47	75.98	30.35	38.45	53.95
Max. value (AU/mL)	8.377	18.39	28.53	94.33	36.84	48.08	62.42

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 6 samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Reproducibility	10	11	12	13	14	15	Positive control
Number of determinations	90	90	90	90	90	90	90
Mean (AU/mL)	7.151	15.87	24.16	83.39	32.17	41.74	58.01
Standard deviation	0.294	0.673	1.136	3.115	1.204	1.567	1.193
Coefficient of variation (%)	4.1	4.2	4.7	3.7	3.7	3.8	2.1
Min. value (AU/mL)	6.652	14.49	22.47	75.98	30.35	38.45	53.95
Max. value (AU/mL)	8.377	18.39	28.53	94.33	36.84	48.08	62.42

15.2.4. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than actually present. However, a well-optimized two-step method excludes grossly underestimated results because the analytical signals remain consistently high (saturation curve).

Analysis of saturation effect was evaluated by testing four high-titred serum samples positive for *Borrelia burgdorferi* IgM. All samples resulted in estimated concentration values above the assay range that would be expected with high-titred samples, indicating no sample misclassification.

15.3. LIAISON® QuantiFERON® LymeDetect® (REF 311034)

15.3.1. 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® LymeDetect® assay. The testing was based on CLSI document EP07.

Substances	**Tested concentration**	**Substances**	**Tested concentration**
Triglycerides	3000 mg/dL	IL-2	10 ng/mL
Haemoglobin	1000 mg/dL	IL-4	5 ng/mL
Unconjugated bilirubin	40 mg/dL	IL-5	100 ng/mL
Conjugated bilirubin	40 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)	120 IU/mL	IFN-alpha	50 ng/mL
HAMA	600 ng/mL	IFN-beta	50 ng/mL
Cholesterol	500 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

15.3.2. Precision with LIAISON® XL Analyzer

A within-laboratory precision study was performed consulting CLSI document EP05-A3 in the preparation of the testing protocol. Ten (10) Li-Heparin samples containing spiked concentrations of native IFN-γ spanning the assay range were assayed in duplicate, in 2 runs per day, over 20 operating days with multiple technicians, by 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

15.3.3. 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® LymeDetect®. 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 spanning 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, standard deviation, and 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

15.3.4. High-dose hook effect

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

16. CLINICAL PERFORMANCE

Clinical performance of LIAISON® LymeDetect® assay was established with specimens collected at five European clinical sites from Lyme Borreliosis patients with and without signs of Erythema Migrans (EM), and from healthy subjects.

Three hundred and nine (309) samples were prospectively collected from subjects over the course of the study during the Lyme Borreliosis season. The enrollment of Lyme patients (123) and healthy subjects (186) has been performed in Italy, Germany, Czech Republic, Poland and France.

Diagnostic sensitivity.

One hundred and twenty-three (123) Lyme patients were recruited in endemic areas and tested with the LIAISON® LymeDetect® assay and by the diagnostics standard of care for Borreliosis, i.e. standard Two-Tiered Test (sTTT).

One hundred and five (105) out of one hundred and twenty-three (123) patients did have evidence of Erythema Migrans appearance, while eighteen (18) out of one hundred and twenty-three (123) patients did not show signs of Erythema Migrans, with a rate of 14.6% (18/123).

After testing with LIAISON® LymeDetect® assay, Lyme patients were classified into two categories: within 21 days from the appearance of evidence of infection (i.e. appearance of the Erythema Migrans or from the tick bite) and after more than 21 days. Diagnostic sensitivity obtained with the LIAISON® LymeDetect® assay is shown in the table below, also in comparison with the sTTT algorithm.

	LymeDetect® Algorithm		sTTT Algorithm	
	Diagnostic Sensitivity	Wilson 95% CI	Diagnostic Sensitivity	Wilson 95% CI
≤ 21 days	73.53% (50/68)	61.99% - 82.55%	48.53% (33/68)	37.05% - 60.17%
> 21 days	81.82% (45/55)	69.67% - 89.81%	67.27% (37/55)	54.10% - 78.19%

Diagnostic Specificity

Diagnostic specificity was evaluated with the LIAISON® LymeDetect® assay on healthy subjects.

	LymeDetect® Algorithm	
	Diagnostic Specificity	Wilson 95% CI
Healthy subjects	100.0% (186/186)	97.98% - 100%

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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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