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Deletions: -

LIAISON® Borrelia IgG (REF 310880)

1. INTENDED PURPOSE

The LIAISON® Borrelia IgG assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination of specific IgG antibodies to *Borrelia burgdorferi sensu lato (*including strains *Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia afzelii)* in human serum, plasma or cerebrospinal fluid (CSF) samples. The assay is intended as an aid in the diagnosis of recent, acute or past *Borrelia burgdorferi sensu lato* infection, in subjects with clinical evidences of skin lesions due to suspected tick bite, neurological disorders or arthritis, or whenever a Borrelia infection may be suspected.
The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Lyme Borreliosis (LB) or Borrelia burgdorferi sensu lato infection is the most common vectorborne disease in temperate zones of the northern hemisphere[1]. LB is transmitted to humans during the blood feeding of ticks of the genus Ixodes: in Europe mainly Ixodes ricinus, and to a lesser extent I. persulcatus. The symptoms of LB were described almost a century ago by the Swedish dermatologist Arvid Afzelius, but the disease was not identified until 1977, in the area of Lyme (Connecticut) in the United States – hence the name Lyme disease. Following the discovery in 1982 of the spirochete (spiral-shaped bacterium) Borrelia burgdorferi s.l. as the causative agent of LB, the disease emerged as the most prevalent arthropod-borne infection in northern temperate climate zones around the world[1]. Spirochetes are maintained in nature in ticks and in the blood of certain animal species: in Europe particularly insectivores, small rodents, hares and birds.

Lyme borreliosis is an inflammatory multi-organ disease that is treatable with antibiotics[2]. Neither subclinical nor symptomatic infections provide immunity. It manifests itself initially as a localised infection of the skin called erythema migrans, which occurs in about 60–80% of cases within 2–30 days of a tick bite and consists of a red skin rash or lesion spreading from the site of the bite. Because of its light symptoms, this early-stage inflammation of the skin can be overlooked or not even be visible. If left untreated, a disseminated infection that affects the nervous system, joints and/or the heart may follow within days or weeks. The disease progresses very differently depending on the individual.

If the late manifestations remain untreated for a long period of time, there is a higher risk of the patient having persistent physical symptoms and of their skin, joints and nervous system not properly healing[2].

Diagnosis of Lyme borreliosis is based on a complete diagnostic workup, including medical history with compatible clinical symptoms, objective signs, possible exposure to tick bites, and exclusion of other diseases, not laboratory testing alone[5].

Detection of antibodies to B. burgdorferi is currently the laboratory method of choice in a routine clinical setting. Infection with B. burgdorferi induces an immune response with clinical findings, such as skin lesions, neurological signs, cardiac involvement (e.g. atrioventricular block), or arthritis involving the large joints[5]. Detection of specific IgG and IgM antibodies is recommended for routine laboratory testing for Lyme borreliosis[2]. Diagnostic use of very sensitive early-phase antigens, such as VlsE, enables the detection of a specific IgG response very early on in the course of the infection[2,3,4]. When Lyme neuroborreliosis is suspected, the CSF should be examined for signs of inflammation and intrathecal antibody production (antibody index, AI) to B. burgdorferi determined by analysing paired serum and CSF samples obtained on the same day[5,6,7].

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative 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.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with *Borrelia burgdorferi* VIsE (*Borrelia garinii* strain pBi) recombinant antigen (approx.100 µg/mL) (obtained in *E. coli*), 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		100

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

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® 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® Module (REF 319130). – – LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300). LIAISON® Light Check 12 (REF 319150). LIAISON® Wash/System Liquid (REF 319100). LIAISON® Waste Bags (REF 450003). LIAISON® Cleaning Kit (REF 310990).

LIAISON® XS Analyzer
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® Borrelia IgG controls (negative and positive) (REF 310881).
LIAISON® Borrelia IgG Liquor controls (negative and positive) (REF 310882).

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 serum and plasma units 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. The waste must be handled with care and disposed of in compliance with the 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 use kits or components beyond the expiration date given on the label.

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

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

7. REAGENT PREPARATION

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 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, calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foam 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 integral into the reagent area

LIAISON® Analyzer

– Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS analyzers

– LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device which 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. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

CONTROLS

Refer to the LIAISON® Borrelia IgG and Borrelia IgG Liquor Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY

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

9. SPECIMEN COLLECTION AND PREPARATION

Serum or plasma samples.

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

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 5 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 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 is 170 µL of specimen (20 µL specimen + 150 µL dead volume). No further manipulation is required, because the instrument automatically dilutes specimens before testing.

Cerebrospinal fluid samples

Liquor should be collected aseptically by lumbar puncture on the same day as serum or plasma.

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.

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 5 freeze-thaw cycles, however multiple freeze thaw cycles should be avoided.

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

Samples having particulate matter, turbidity, or erythrocyte debris may require clarification by filtration or centrifugation before testing.

Grossly haemolyzed samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Excessive blood contamination of the specimens may lead to false positive results. Check for and remove air bubbles before assaying.

The minimum volume required is 200 µL specimen (50 µL specimen + 150 µL dead volume). No further manipulation is required, because the instrument automatically dilutes specimens before testing.

10. CALIBRATION

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows eight 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 one (1) week before.
– Each time a new lot of integral is used.
– Control values lie outside the expected ranges.
– LIAISON® and LIAISON® XL analyzers: 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® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL and LIAISON® XS Analyzers: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

This test requires the following assay files: Bor-Gc, Bor-G and BoGCSF.

To test specimens use Bor-G or BoGCSF.

Never use Bor-Gc.

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

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label 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.

LIAISON® XL and LIAISON® XS analyzers. 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.

Assay file for *Borrelia burgdorferi* IgG determination in serum or plasma samples is **Bor-G**.
Assay file for *Borrelia burgdorferi* IgG determination in cerebrospinal fluid samples is **BoGCSF**.

The analyzer operations are as follows:

Serum or plasma specimens	Cerebrospinal fluid specimens
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.	1. Dispense coated magnetic particles and diluent. 2. Dispense 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.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Control Borrelia IgG (REF 310881) and LIAISON® Borrelia IgG Liquor controls (REF 310882)
(a) at least once per day of use,
(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) to assess adequacy of performance of the open integral beyond four weeks, or in agreement with 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 they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS IN SERUM OR PLASMA

13.1. Borrelia IgG test (serum or plasma samples)

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®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 5 to 240 AU/mL *Borrelia burgdorferi* IgG.
Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:10). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows up to 10 sample predilutions to be performed.

Dilution of over-range specimens may not provide accurate quantitative measurements due to sample-dependent unparallel response.

Sample results should be interpreted as follows:
Samples with *Borrelia burgdorferi* IgG concentrations below 10 AU/mL should be graded *negative*.
Samples with *Borrelia burgdorferi* IgG concentrations ranging between 10 and 15 AU/mL should be graded *equivocal*. *Equivocal samples must be retested in order to confirm the initial result. Samples which are positive at the second test should be considered positive. Samples which are negative at the second test should be considered negative. A second sample should be collected and tested no less than one week later when the result is repeatedly equivocal.*
Samples with *Borrelia burgdorferi* IgG concentrations equal to or above 15 AU/mL should be graded *positive*.

13.2. Interpretation of results for serum or plasma samples

A negative result for IgM and/or IgG antibodies to *Borrelia burgdorferi* generally indicates that the patient has not been infected, but does not always rule out acute borreliosis, because the infection may be in its very early stage and the patient may be still unable to synthesize *Borrelia burgdorferi* specific antibodies, or the antibodies may be present in undetectable levels. Specific IgM antibodies are more easily detected in the early stages of infection; in later stages they progressively decline. It should be underlined that the test scores negative during the first weeks after infection. If clinical exposure to *Borrelia burgdorferi* is suspected despite a negative or equivocal finding, a second sample should be collected and tested for IgM and IgG later during the course of infection.

A positive result for IgM and/or IgG antibodies to *Borrelia burgdorferi* generally indicates exposure to the pathogen (acute or past infection). A single specimen, however, can only help estimate the serological status of the individual. An isolated positive IgM result is observed relatively often in the early stages of the disease, but rarely in the later stages. An isolated positive IgG result may indicate either active Lyme disease or past infection with persisting antibodies. The following table summarizes the different immunological pictures. Results were obtained using LIAISON® Borrelia assays.

Borrelia burgdorferi IgM result	*Borrelia burgdorferi* IgG result	Interpretation
negative	negative	No evidence of infection. In case of clinical uncertainty (presence of tick bite or neurological symptoms), the patients should be followed up during time.
positive	negative	Probable infection at an early stage.
negative	positive	Probable infection at any stage.
positive	positive	Probable acute infection.

14. INTERPRETATION OF RESULTS IN CEREBROSPINAL FLUID

14.1. Borrelia IgG test (cerebrospinal fluid samples)

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®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 0.2 to 240 AU/mL *Borrelia burgdorferi* IgG.
Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested. The recommended dilution factor is 1:10; when the diluted samples still score above the assay range, the test should be repeated after prediluting the samples 1:100. The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows up to 10 sample predilutions to be performed.

14.2. Interpretation of results for cerebrospinal fluid samples

Sample results could be interpreted as follows (CSF matrix specific interpretation):
Samples with *Borrelia burgdorferi* IgG concentrations below 4.5 AU/mL should be graded *negative*.
Samples with *Borrelia burgdorferi* IgG concentrations ranging between 4.5 and 5.5 AU/mL should be graded *equivocal*. *Equivocal samples must be retested in order to confirm the initial result. Samples which are positive in the second test should be considered positive. Samples which are negative in the second test should be considered negative. A second sample should be collected and tested no less than one week later when the result is repeatedly equivocal.*
Samples with *Borrelia burgdorferi* IgG concentrations equal to or above 5.5 AU/mL should be graded *positive*.

A negative result for IgG antibodies to *Borrelia burgdorferi* indicates unlikely intrathecal synthesis of *Borrelia burgdorferi* antibodies. If neuroborreliosis is strongly suspected despite a negative finding, further diagnostic investigation is suggested.

A positive result for IgG antibodies to *Borrelia burgdorferi* suggests possible intrathecal synthesis of *Borrelia burgdorferi* antibodies: neuroborreliosis is therefore suspected.
Positive results may be observed in patients with extremely high levels of circulating *Borrelia burgdorferi* antibodies as well as in patients positive for *Borrelia burgdorferi* serum antibodies associated with high albumin concentrations in cerebrospinal fluid. The latter finding suggests possible damage to the blood/cerebrospinal fluid barrier.

For more reliable quantification of intrathecal immunoglobulin synthesis, sample results should be interpreted by referring to the specific CSF/serum Antibody Index. *The specific detection of antibodies to Borrelia burgdorferi* in CSF, together with neurological symptoms of borreliosis, has a strong association with the diagnosis of neuroborreliosis. The intrathecal presence of specific *Borrelia burgdorferi* antibodies can be determined as Antibody Index (AI) through the analysis of a blood sample and a CSF sample concomitantly collected from the same patient. This Index is used to differentiate between active intrathecal synthesis and passive diffusion of *Borrelia burgdorferi* specific IgG from serum to CSF. The AI is evaluated taking into due consideration the total IgG concentration in patients' serum (Reiber method) and CSF, in the same two samples (pair of serum and CSF simultaneously collected from the same patient) where the Borrelia IgG determination is carried out.

The specific CSF/serum Antibody Index can be determined by the formula:

$$\text{Antibody Index} = \frac{\text{LIAISON® Borrelia IgG CSF / LIAISON® Borrelia IgG serum}}{\text{(CSF Total IgG titer / serum Total IgG titer) * 10}^{a}}$$

According to reference (Lange P et al, poster presented in ECCMID Barcelona Spain 2008), the normal AI range is from 0.7 to 1.3, provided that Borrelia-specific antibodies are present in the serum and there is no intrathecal synthesis.

Values above 1.5 generally indicate synthesis of Borrelia specific antibodies in the CNS. Values below 0.7 or between 1.3 and 1.5 are inconclusive and the test should be repeated with a further pair of samples.

The output of the calculation formula when LIAISON Borrelia doses are graded negative by using matrix specific interpretation of results should be interpreted with care. Values of Antibody Index below 0.7 may be due to the set specimen dilutions used for testing. CSF matrix specific interpretation is recommended to solve the diagnostic classification for these inconclusive results.

The total IgG test method used, and the titer obtained, should be validated by the laboratory. The same method should be used to titer the total IgG in both the blood and the CSF. When the total IgG titer is close to zero, the formula should not be applied because of unreliable calculations.

The above calculation formula should be validated for use by the laboratory prior to defining the diagnosis of neuroborreliosis.

[a]The cerebrospinal fluid to serum volume invariable ratio used in the LIAISON® Borrelia IgG test is 10.

15. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® Borrelia test is used in conjunction with other manufacturers' assays for detection of specific *Borrelia burgdorferi* serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- Test results are reported quantitatively as positive or negative for the presence of *Borrelia burgdorferi* IgG. However, diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgement.
- Pre-diluted CSF specimens may lead to inconclusive results due to technical limitation related to the set specimen dilution used in the test.
- Antibiotic therapy during the early stages of the disease often prevents development of antibody response.
- Results obtained with LIAISON® Borrelia IgG assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- Integrals may not be exchanged between analyzer types (LIAISON®, 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.

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1. 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 20 mg/dL bilirubin), or by freeze-thaw cycles of samples.
Controlled studies of potentially interfering substances or conditions showed that the assay performance in cerebrospinal fluid samples was not affected by haemolysis (up to 1000 mg/dL haemoglobin) 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

16.2. Precision with LIAISON® Analyzer (serum or plasma samples)

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

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

Repeatability	H	I	J	K
Number of determinations	20	20	20	20
Mean (AU/mL)	1.6	36.4	63.0	135.1
Standard deviation (AU/mL)	0.18	2.54	2.28	8.64
Coefficient of variation (%)	10.9	7.0	3.6	6.4
Min. value (AU/mL)	1.4	32.5	59.7	120.3
Max. value (AU/mL)	2.1	41.4	69.0	158.9

Reproducibility. Twenty replicates were performed in different days (two or three runs per day) to evaluate reproducibility, using the same instrument.

Reproducibility	H	L	M	N
Number of determinations	20	20	20	20
Mean (AU/mL)	1.7	16.8	52.9	87.1
Standard deviation (AU/mL)	0.29	1.67	5.10	8.64
Coefficient of variation (%)	16.9	10.0	9.7	9.9
Min. value (AU/mL)	1.3	13.9	43.6	71.0
Max. value (AU/mL)	2.5	20.4	64.1	104.9

Lot-to-Lot Reproducibility. Samples tested in singleton on five (5) different LIAISON instruments on four (4) different batches.

	LIAISON® Borrelia IgG (Code 310880) on Serum samples on LIAISON®					
Sample ID	A	B	C	D	Positive control	*Negative control
Mean (AU/mL/RLUs)	135.78	16.75	76.11	40.36	31.27	674.5
Inter-lot coefficient of variation (%)	4.0	3.4	4.2	5.9	7.9	33.8

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

16.3. Precision with LIAISON® Analyzer (cerebrospinal fluid samples)

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

Repeatability. Ten replicates were performed in the same run to evaluate in-house repeatability.

Repeatability	1	2	3	4	5	6
Number of determinations	10	10	10	10	10	10
Mean (AU/mL)	2.77	8.64	35.04	99.54	148.94	195.38
Standard deviation (AU/mL)	0.05	0.23	0.75	4.30	6.06	9.04
Coefficient of variation (%)	1.74	2.68	2.13	4.32	4.07	4.63
Min. value (AU/mL)	2.70	8.20	34.00	94.50	141.10	176.70
Max. value (AU/mL)	2.80	9.00	36.50	106.30	157.80	210.80

Reproducibility. Ten replicates were performed in five different days (two runs per day) to evaluate reproducibility, using the same instrument.

Reproducibility	1	2	3	4	5	6
Number of determinations	10	10	10	10	10	10
Mean (AU/mL)	3.10	9.10	33.27	89.51	146.64	175.57
Standard deviation (AU/mL)	0.54	0.69	3.39	9.39	11.36	15.94
Coefficient of variation (%)	17.34	7.56	10.18	10.49	7.75	9.08
Min. value (AU/mL)	2.10	7.60	26.90	73.40	131.30	145.20
Max. value (AU/mL)	3.50	9.70	36.70	106.50	165.40	198.80

Lot-to-Lot Reproducibility. Samples tested in singleton on five (5) different LIAISON instruments on four (4) different batches.

	LIAISON® Borrelia IgG (Code 310880) on CSF samples on LIAISON®					
Sample ID	A	B	C	D	Positive control	*Negative control
Mean (AU/mL/RLUs)	2.88	148.35	76.66	16.58	48.865	331.8
Inter-lot coefficient of variation (%)	7.8	3.0	5.9	2.3	6.8	20.0

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

16.4. Precision with LIAISON® XL Analyzer (serum or plasma samples)

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	Negative control	Positive control
Number of determinations	20	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	0.7324	31.70
Standard deviation	0.37	1.51	2.34	2.98	2.58	8.69	10.90	0.056	1.12
Coefficient of variation (%)	5.0	6.6	9.4	7.0	4.9	9.9	10.2	7.6	3.5
Min. value	6.280	19.76	17.79	35.74	48.55	59.36	80.08	0.5898	29.53
Max. value	7.844	25.45	27.33	47.12	59,04	96.45	118.0	0.8157	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	Negative control	Positive control
Number of determinations	20	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	0.9386	34.66
Standard deviation	0.41	2.49	1.97	3.00	5.10	11.70	15.35	0.10	2.57
Coefficient of variation (%)	5.5	8.8	6.8	6.4	8.3	12.4	13.2	11.1	7.4
Min. value	6.592	23.99	25.90	41.44	53.59	69.30	77.96	0.7476	30.84
Max. value	7.959	32.79	34.52	52.23	70.10	125.3	140.5	1.097	39.61

Lot-to-Lot Reproducibility. Samples tested in singleton on five (5) different LIAISON instruments on four (4) different batches.

	LIAISON® Borrelia IgG (Code 310880) on Serum samples on LIAISON® XL					
Sample ID	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.

16.5. Precision with LIAISON® XL Analyzer (cerebrospinal fluid samples)

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	Negative control	Positive control
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	10.82	21.70	138.1	328.9	0.01491	49.37
Standard deviation (AU/mL)	0.42	1.29	16.37	23.96	0.020	1.80
Coefficient of variation (%)	3.9	5.9	11.8	7.0	133.4	3.6
Min. value (AU/mL)	9.822	18.19	96.61	283.1	0.0000	46.48
Max. value (AU/mL)	11.34	23.14	158.2	366.9	0.08103	53.46

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

Reproducibility	1	2	3	4	Negative control	Positive control
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	12.05	22.58	135.8	268.1	0.1096	56.54
Standard deviation (AU/mL)	1.38	2.12	34.09	12.53	0.060	3.97
Coefficient of variation (%)	11.5	9.4	25.1	4.7	55.0	7.0
Min. value (AU/mL)	10.14	18.56	64.03	246.0	0.02772	49.77
Max. value (AU/mL)	15.19	25.93	193.9	295.0	0.2496	61.97

Lot-to-Lot Reproducibility. Samples tested in singleton on five (5) different LIAISON instruments on four (4) different batches.

	LIAISON® Borrelia IgG (Code 310880) on CSF samples on LIAISON® XL					
Sample ID	A	B	C	D	Positive control	*Negative control
Mean (AU/mL/RLUs)	2.882	166.55	84.16	18.145	52.323	781.2
Inter-lot coefficient of variation (%)	10.3	17.1	5.3	10.3	3.5	6.4

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

16.6. Precision with LIAISON® XS Analyzer (serum or plasma samples)

A five day precision study was conducted on three LIAISON® XS Analyzers to verify the precision with the LIAISON® Borrelia IgG assay. 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 LIAISON® Control Borrelia IgG set was also included in the five day 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 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	8	9	10	11	12	13	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (AU/mL)	6.913	9.812	16.70	29.40	39.94	85.14	1147	29.52
Standard deviation	0.161	0.217	0.517	0.680	0.932	2.657	35.73	0.746
Coefficient of variation (%)	2.3	2.2	3.1	2.3	2.3	3.1	3.1	2.5
Min. value	6.038	8.913	12.89	26.52	36.30	74.50	1024	25.58
Max. value	7.668	10.80	17.64	31.02	43.34	92.79	1324	32.14

*Negative Control is expressed in RLU because out of the Assay Range.

Reproducibility.

Reproducibility	8	9	10	11	12	13	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (AU/mL)	6.913	9.812	16.70	29.40	39.94	85.14	1147	29.52
Standard deviation	0.190	0.263	0.593	0.772	1.104	3.009	60.38	1.001
Coefficient of variation (%)	2.7	2.7	3.6	2.6	2.8	3.5	5.3	3.4
Min. value	6.038	8.913	12.89	26.52	36.30	74.50	1024	25.58
Max. value	7.668	10.80	17.64	31.02	43.34	92.79	1324	32.14

*Negative Control is expressed in RLU because out of the Assay Range.

16.7. Precision with LIAISON® XS Analyzer (cerebrospinal fluid samples)

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

A coded panel comprised of 3 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 LIAISON® Control Borrelia IgG set was also included in the five day 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 value, standard deviation, and coefficient of variation of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability.

Repeatability	5	6	7	Negative control*	Positive control
Number of determinations	90	90	90	90	90
Mean (AU/mL)	7.055	32.23	108.7	400	51.06
Standard deviation (AU/mL)	0.148	0.866	5.901	18.15	1.764
Coefficient of variation (%)	2.1	2.7	5.4	4.5	3.5
Min. value (AU/mL)	6.403	27.33	71.37	357	42.14
Max. value (AU/mL)	7.536	34.19	122.5	462	55.43

*Negative Control is expressed in RLU because out of the Assay Range.

Reproducibility.

Reproducibility	5	6	7	Negative control*	Positive control
Number of determinations	90	90	90	90	90
Mean (AU/mL)	7.055	32.23	108.7	400	51.06
Standard deviation (AU/mL)	0.201	0.961	6.280	24.27	2.080
Coefficient of variation (%)	2.9	3.0	5.8	6.1	4.1
Min. value (AU/mL)	6.403	27.33	71.37	357	42.14
Max. value (AU/mL)	7.536	34.19	122.5	462	55.43

*Negative Control is expressed in RLU because out of the Assay Range.

16.8. Trueness (serum or plasma samples)

5 samples were prepared from a serum at high level of analyte and a serum at low level of analyte (near to the assay range extremes) mixing them together in different proportions in order to obtain analyte levels evenly distributed along the assay range according to following ratio: negative neat NS1 (100%), negative + high level in ratio 2:1 (66.7:33.3%), negative + high level in ratio 1:1 (50:50%), negative + high level in ratio 1:2 (33.3:66.7%) and a high level neat PS1 (100%). The obtained samples were tested in five replicates each on one lot and on one instrument. Percent recoveries were determined through calculation of recovery % for each dilution point as follows:

% Recovery = (mean obtained conc.)/(expected conc.)*100.

Measured versus expected Borrelia IgG concentrations were analyzed by linear regression.

The correlation coefficient (r) was = 0.998 and the slope = 0.998.

Sample	Low (%)	High (%)	Expected (AU/mL)	Measured (AU/mL)	Recovery (%)
BORG-S1	100	-	5.63	5.628	-
BORG-S2	66.7	33.3	66.92	73.83	110.3
BORG-S3	50	50	98.51	102.4	103.9
BORG-S4	33.3	66.7	129.54	135.5	104.6
BORG-S5	-	100	191.40	191.4	-
				Average Recovery (%)	106.3

16.9. Linearity (serum or plasma samples)

The assay linearity for LIAISON® Borrelia IgG test has been checked by the dilution test.

Dilution test. Four serum samples containing high *Borrelia burgdorferi* IgG concentrations were tested as such and after serially diluting with the specimen diluent. *Borrelia burgdorferi* IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.995 to 0.999.

Dilution	Expected concentration, AU/mL	Measured concentration, AU/mL	% Recovery	Dilution	Expected concentration, AU/mL	Measured concentration, AU/mL	% Recovery
neat	–	112.5	–	neat	–	144.3	–
1:2	56.3	43.8	78.0	1:2	72.2	60.6	84.0
1:4	28.1	23.9	85.0	1:4	36.1	32.1	89.0
1:8	14.1	13.7	97.0	1:8	18.0	19.7	109.0
1:16	7.0	6.4	91.0	1:16	9.0	7.7	85.0
1:32	3.5	2.7	77.0	1:32	4.5	3.0	67.0
neat	–	176.6	–	neat	–	184.0	–
1:2	88.3	99.0	112.0	1:2	92.0	84.4	92.0
1:4	44.2	45.4	103.0	1:4	46.0	48.8	106.0
1:8	22.1	22.4	101.0	1:8	23.0	24.4	106.0
1:16	11.0	9.9	90.0	1:16	11.5	13.7	119.0
1:32	5.5	4.4	80.0	1:32	5.8	6.4	111.0
				1:64	2.9	2.6	90.0

16.10. Trueness (cerebrospinal fluid samples)

5 samples were prepared from a cerebrospinal fluid (CSF) specimen at high level of analyte and a cerebrospinal fluid (CSF) specimen at low level of analyte (near to the assay range extremes) mixing them together in different proportions in order to obtain analyte levels evenly distributed along the assay range according to following ratio: negative neat NS1 (100%), negative + high level in ratio 2:1 (66.7:33.3%), negative + high level in ratio 1:1 (50:50%), negative + high level in ratio 1:2 (33.3:66.7%) and a high level neat PS1 (100%).

The obtained samples were tested in five replicates each on one lot and on one instrument.

Percent recoveries were determined through calculation of recovery % for each dilution point as follows:

% Recovery= (mean obtained conc.)/(expected conc.)*100.

Measured versus expected Borrelia IgG concentrations were analyzed by linear regression.

The correlation coefficient (r) was = 0.985 and the slope = 1.03.

Sample	Low (%)	High (%)	Expected (AU/mL)	Measured (AU/mL)	Recovery (%)
BORGCSF-S1	100	-	3.81	3.808	-
BORGCSF-S2	66.7	33.3	84.4	84.84	100.5
BORGCSF-S3	50	50	108.9	120.0	110.2
BORGCSF-S4	33.3	66.7	144.0	167	116.0
BORGCSF-S5	-	100	214.0	214	-
				Average Recovery (%)	108.9

16.11. Linearity (cerebrospinal fluid samples)

The assay linearity for LIAISON® Borrelia IgG test has been checked by the dilution test.

Dilution test. Four cerebrospinal fluid samples containing high *Borrelia burgdorferi* IgG concentrations were tested as such and after serially diluting with the specimen diluent. *Borrelia burgdorferi* IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.997 to 0.999.

Dilution	Expected concentration, AU/mL	Measured concentration, AU/mL	% Recovery	Dilution	Expected concentration, AU/mL	Measured concentration, AU/mL	% Recovery
neat	–	141.0	–	neat	–	141.9	–
1:2	70.5	78.3	111.1	1:2	71.0	67.2	94.7
1:4	35.3	43.1	122.3	1:4	35.5	31.3	88.2
1:8	17.6	23.0	130.5	1:8	17.7	16.7	94.2
1:16	8.8	12.2	138.4	1:16	8.9	8.0	90.2
1:32	4.4	6.1	138.4	1:32	4.4	3.0	67.7
1:64	2.2	2.2	99.9				
neat	–	184.7	–	neat	–	196.4	–
1:2	92.4	85.1	92.1	1:2	98.2	101.2	103.1
1:4	46.2	40.3	87.3	1:4	49.1	56.8	115.7
1:8	23.1	20.4	88.4	1:8	24.6	26.4	107.5
1:16	11.5	10.1	87.5	1:16	12.3	14.4	117.3
1:32	5.8	4.6	79.7	1:32	6.1	7.0	114.1
				1:64	3.1	2.8	91.2

16.12. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve).
Analysis of saturation effect for LIAISON® Borrelia IgG test was evaluated by testing three high-titred serum samples positive for *Borrelia burgdorferi* IgG as well as four high-titred cerebrospinal fluid 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.

16.13. Analytical and functional sensitivity (serum or plasma samples)

The Limit of Blank (LoB) for the LIAISON® Borrelia IgG assay is 0.138 AU/mL. Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.654 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Borrelia IgG assay is 0.308 AU/mL. Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® Borrelia IgG assay is 0.711 AU/mL.

16.14. Analytical and functional sensitivity (cerebrospinal fluid samples)

The Limit of Blank (LoB) for the LIAISON® Borrelia IgG assay is 0.131 AU/mL. Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.654 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Borrelia IgG assay is 0.264 AU/mL. Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® Borrelia IgG assay is 0.754 AU/mL.

17. EXPECTED VALUES

17.1. Diagnostic specificity and sensitivity (serum or plasma samples)

Diagnostic specificity and sensitivity were estimated by testing 394 specimens from different populations coming from collection centers located in endemic areas (Germany and in Northeast Italy). The specimens were tested with several comparison methods and consensus between them, and the available clinical and serological data were applied to define the expected results.

Diagnostic specificity. 100 routine serum specimens from subjects living in an area endemic for borreliosis were graded negative by reference tests (enzyme immunoassay, Western blot). In the same group of subjects, Borrelia IgG test scored negative, with 98.0% diagnostic specificity (95% confidence interval: 93.0-100%).

Diagnostic sensitivity. 294 serum specimens from patients with clinically characterized Lyme borreliosis were tested with the Borrelia IgG test. The following diagnostic sensitivity data were obtained.

Clinical condition	Number of cases	IgG result	
		% of positive specimens	95% confidence interval
Erythema chronicum migrans	98	66.3	56.1-75.5
Neuroborreliosis	87	90.8	82.7-95.9
Arthritis	109	93.6	87.2-97.4
Total	294	83.7	78.9-87.7

Equivocal results were not taken into consideration for the calculation of diagnostic sensitivity.

17.2. Diagnostic concordance (cerebrospinal fluid samples)

In a clinical study, 209 routine cerebrospinal fluid specimens were tested by the LIAISON® Borrelia IgG test and by a reference enzyme immunoassay. No Antibody Indexes were calculated, but sample results were interpreted following CSF matrix specific interpretation. The specimens were not clinically characterized, but were collected from subjects investigated for suspected *Borrelia burgdorferi* infection living in an area endemic for borreliosis (Germany). Five samples scored positive and 194 scored negative in both tests, two samples scored negative by the comparison enzyme immunoassay and positive by LIAISON®, and five samples scored positive by the comparison enzyme immunoassay and negative by

LIAISON®. Three samples scored equivocal in one or both tests and therefore were not included in the data analysis. Diagnostic concordance was 96.6% (199/206) - 95% confidence interval: 93.1-98.6%.

In another clinical study, 27 cerebrospinal fluid specimens were tested by the LIAISON® Borrelia IgG test and by a reference enzyme immunoassay. The specimens were collected from patients with clinically characterized neuroborreliosis. 26 samples scored positive in both tests and one sample scored negative by the comparison enzyme immunoassay and positive by LIAISON®. Diagnostic concordance was 96.3% (26/27) - 95% confidence interval: 81.0-99.9%.

17.3. Diagnostic concordance by Antibody Index

In a clinical study (Lange P et al, poster presented in ECCMID Barcelona Spain 2008), 90 CSF and serum sample pairs from a routine analysis of a German hospital, were analyzed by the LIAISON® Borrelia IgG and IgM quantitative assays and by a reference quantitative ELISA (CE marked) assay. The specimens were not clinically characterized, but were collected from subjects investigated for suspected Borrelia burgdorferi infection living in an area endemic for borreliosis (Germany). 50 sample pairs showed an increased AI above 1.5 by the reference ELISA method, either for IgG or for IgM. Out of these 50 pairs, 34 were classified concordantly for IgG and IgM Antibody Indexes, by the ELISA and LIAISON® assays (Table 1) while 16 were not (Table 2). For 1 sample pair it was not possible to complete determinations, and thus the pair was excluded from calculations. 39 sample pairs had AI <1.5 or non-detectable Borrelia IgG and IgM concentrations in the CSF, neither with the ELISA reference method nor with LIAISON® Borrelia quantitative assays.

Table 1

AI ELISA		Congruent results between both assays	AI LIAISON®	
IgG +	IgM +	26	IgG +	IgM +
IgG +	IgM -	8	IgG +	IgM -
IgG -	IgM +	0	IgG -	IgM +
IgG -	IgM -	39	IgG -	IgM -

Table 2

AI ELISA		Incongruent results between both assays	AI LIAISON®	
IgG +	IgM +	1	IgG +	IgM -
IgG +	IgM -	10	IgG +	IgM +
IgG +	IgM -	1	IgG -	IgM -
IgG -	IgM +	1	IgG +	IgM +
IgG -	IgM +	1	IgG +	IgM -
IgG -	IgM +	1	IgG -	IgM -
IgG -	IgM -	1	IgG -	IgM +

Based on these results, the Diagnostic Sensitivity obtained for AI determination on serum/CSF sample pairs for the LIAISON® Borrelia IgG assay was 97.8% (45/46, 95% confidence interval: 88.5-99.9%), while the obtained Diagnostic Specificity was 95.3% (41/43, 95% confidence interval: 84.2-99.4%).

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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