DiaSorin Italia S.p.A.
Via Crescentino snc - 13040 Saluggia (VC) - Italy
www.diasorin.com
Tel. +39.0161.4871

CE 0459

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Deletions: -

LIAISON® Borrelia IgM II (REF 310010)

1. INTENDED PURPOSE

The LIAISON® Borrelia IgM II assay uses chemiluminescent immunoassay (CLIA) technology 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 or plasma 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 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.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

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 VlsE (*Borrelia garinii* strain pBi and *Borrelia sensu stricto* strain B31) recombinant antigens (approx. 50 µg/mL) (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. 0.59 Index), 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. 4.15 Index), 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 conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate 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 IgM Quant controls
LIAISON® Borrelia IgM II controls (negative and positive) (REF 310011).

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.

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

Warning - Before removing the seals from the vials and before each calibration, gently shake the reagent integral avoiding foam formation.

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 IgM Quant / Borrelia IgM II 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.
After this period, it is still possible to keep on using the reagent integral provided that the controls are found within the expected ranges.
– Use always the same LIAISON® Analyzer for a reagent integral already opened.
– Use storage rack provided with the LIAISON® 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

The correct specimen type must be used in the assay. Following matrices have been tested and may be used:
– Serum;
– Plasma collected with the following anticoagulant:
.potassium EDTA;
.heparin;
.citrate.

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 in dry ice (frozen), in wet ice (for 2°-8°C) or at room temperature (20°-25°C), by following 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:
– 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;
– Room temperature sample storage 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 10,000 g for 10') 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. Check for and remove air bubbles before assaying.

The minimum volume required for a single determination is 180 µL of specimen (30 µL specimen + 150 µL dead volume).

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 (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 one 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).

Warning - Before removing the seals from the vials and before each calibration, gently shake the reagent integral avoiding foam formation.

11. ASSAY PROCEDURE

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

The analyzer operations are as follows:

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.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Borrelia IgM Quant / Borrelia IgM II controls (REF 310011)
(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

13.1. Borrelia IgM test

The analyzer automatically calculates *Borrelia burgdorferi* IgM levels expressed as index value 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. 0.1 to 6 index value *Borrelia burgdorferi* IgM.

The cut-off value discriminating between the presence and the absence of *Borrelia burgdorferi* IgM has an index value of 1.0. Sample results should be interpreted as follows:
Samples with *Borrelia burgdorferi* IgM levels below an index value of 0.9 should be graded *negative*.
Samples with *Borrelia burgdorferi* IgM levels ranging between an index value of 0.9 and 1.1 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* IgM levels equal to or above an index value of 1.1 should be graded *positive*.

13.2. Interpretation of results

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. LIMITATIONS OF THE PROCEDURE

– 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 qualitatively as positive or negative for the presence of *Borrelia burgdorferi* IgM. 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.
– Antibiotic therapy during the early stages of the disease often prevents development of antibody response.
– The presence of rheumatoid factor and infectious mononucleosis must be excluded in patients with 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.
– Results obtained with LIAISON® Borrelia IgM II assay may not be used interchangeably with values obtained with different manufacturers' assay methods for detection of specific *Borrelia burgdorferi* serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.
– 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.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. 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 was not affected by anticoagulants (sodium citrate, potassium 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.

Cross-reactions. As a rule, the presence of potentially cross-reactive antibodies 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/equivocal 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. Precision with LIAISON® 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 results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

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

Repeatability	A	B	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.571	1.45	0.145	2.27
Standard deviation	0.026	0.081	0.010	0.16
Coefficient of variation (%)	4.7	5.6	7.3	7.1
Min. value	0.519	1.20	0.127	2.00
Max. value	0.610	1.56	0.163	2.55

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

Reproducibility	A	B	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.654	1.53	0.163	2.59
Standard deviation	0.065	0.087	0.017	0.21
Coefficient of variation (%)	9.9	5.7	10.3	8.0
Min. value	0.531	1.35	0.113	2.21
Max. value	0.818	1.69	0.193	2.94

Lot to lot reproducibility. Twenty replicates were performed in different days (one or two runs per day) with three different lots of integral to evaluate reproducibility.

Reproducibility	LIAISON® Borrelia IgM II (Code 310010) on LIAISON®						
Sample ID	A	B	C	D	E	Negative Control	Positive Control
Mean (Index)	0.64	0.65	0.95	1.31	2.94	0.15	3.41
Standard Deviation	0.06	0.05	0.06	0.12	0.40	0.01	0.31
Inter-lot coefficient of variation (%)	8.7	7.1	6.4	9.3	13.4	9.7	9.2

15.3. 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	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.537	1.50	0.107	2.05
Standard deviation	0.012	0.038	0.0031	0.090
Coefficient of variation (%)	2.2	2.6	2.9	4.4
Min. value	0.510	1.44	0.0999	1.89
Max. value	0.555	1.58	0.112	2.24

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

Reproducibility	1	2	Negative control	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.608	1.51	0.125	2.10
Standard deviation	0.046	0.11	0.0062	0.15
Coefficient of variation (%)	7.6	7.6	5.0	7.1
Min. value	0.496	1.24	0.113	1.77
Max. value	0.723	1.69	0.132	2.32

15.4. Precision with LIAISON® XS Analyzer

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

A coded panel comprised of 7 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 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 Index 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. Ninety replicates were performed in the same test to evaluate repeatability. 7 serum 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	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.326	0.660	0.842	1.43	1.19	1.12	2.13	0.130	1.65
Standard deviation	0.007	0.013	0.020	0.034	0.039	0.030	0.064	0.003	0.056
Coefficient of variation (%)	2.1	2.0	2.4	2.4	3.3	2.7	3.0	2.7	3.4
Min. value	0.298	0.611	0.762	1.31	1.03	1.00	1.81	0.113	1.42
Max. value	0.346	0.697	0.925	1.55	1.28	1.20	2.32	0.144	1.75

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 7 serum 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	3	4	5	6	7	8	9	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.326	0.660	0.842	1.43	1.19	1.12	2.13	0.130	1.65
Standard deviation	0.011	0.017	0.028	0.046	0.045	0.036	0.072	0.007	0.059
Coefficient of variation (%)	3.4	2.6	3.3	3.2	3.8	3.2	3.4	5.3	3.6
Min. value	0.298	0.611	0.762	1.31	1.03	1.00	1.81	0.113	1.42
Max. value	0.346	0.697	0.925	1.55	1.28	1.20	2.32	0.144	1.75

15.5. 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 IgM II test 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.

16. EXPECTED VALUES

16.1. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were estimated by testing 229 specimens from different populations coming from collection centers located in endemic areas (Germany). 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. 88 routine serum specimens from subjects living in an area endemic for borreliosis were graded negative by reference tests (enzyme immunoassay, immunoblot). In the same group of subjects, the LIAISON® Borrelia IgM II test scored negative in 88 out of 88 specimens, with 100% diagnostic specificity (95% confidence interval: 95.9-100%).

Diagnostic sensitivity. 141 serum specimens from patients with clinically characterized Lyme borreliosis were tested in parallel with LIAISON® Borrelia IgM II and IgG tests. The following diagnostic sensitivity data were obtained.

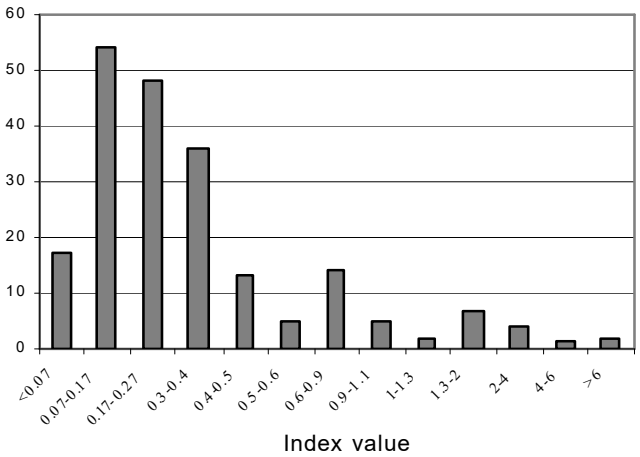
Clinical condition	Number of cases	IgM result		IgG result		IgM + IgG result	
		% positive	95% CI	% positive	95% CI	% positive	95% CI
Erythema chronicum migrans	45	46.7	31.6-62.2	80.0	65.4-90.4	88.9	75.9-96.3
Neuroborreliosis	57	43.9	30.7-57.7	93.0	83.0-98.1	96.5	87.9-99.6
Arthritis	39	25.6	13.0-42.1	97.4	86.5-99.9	97.4	86.5-99.9
Total	141	39.7	31.6-48.3	90.1	83.9-94.5	94.3	89.1-97.5

Equivocal results were not taken into consideration for the calculation of diagnostic sensitivity.
% positive = percentage of positive specimens; 95% CI = 95% confidence interval.

16.2. Prospective study

In a clinical study, 207 routine serum specimens were tested by the LIAISON® Borrelia IgM II test and by an IgM enzyme immunoassay. The immunoblot assay showed discordant results.
The specimens were collected in Germany from subjects investigated for suspected *Borrelia burgdorferi* infection living in an area endemic for borreliosis. The following clinical patterns were observed. The distribution of the prospective population is illustrated in the graph below.

Pattern of *Borrelia burgdorferi* IgM results		No. of specimens graded (grey zone: 0.9-1.1 index)	Immunoblot result, No.		
LIAISON® result	ELISA result		Negative	Equivocal	Positive
negative	negative	165	–	–	–
positive	positive	11	1	2	8
equivocal	equivocal	1	0	1	0
negative	positive	11	6	5	0
equivocal	positive	0	–	–	–
equivocal	negative	3	3	0	0
positive	negative	5	2	2	1
positive	equivocal	0	–	–	–
negative	equivocal	11	9	0	2
Total number of specimens tested		207	21	10	11



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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