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Deletions: §14;

LIAISON® Treponema Screen (REF 310840)

1. INTENDED PURPOSE

The LIAISON® Treponema Screen assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of antibodies to *Treponema pallidum* in human serum and plasma samples included specimens collected post-mortem (non-heart beating). The assay is intended as an aid in the diagnosis of Syphilis infection in individuals with or without symptoms of Syphilis. It is also intended as a screening test for blood and hemocomponents donors as well as for organ, tissue and cells post-mortem donors.
The assay has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

The Treponema pallidum subspecies (subsp.) pallidum is a member of the Gram-negative motile microaerophilic Spirochaetaceae[1] family. It is the causative agent of venereal syphilis[2], a sexually transmitted disease (STD) that can have very serious complications when left untreated.

The course of a syphilis infection follows several stages (primary, secondary, latent, and tertiary), with different signs and symptoms associated with each stage[3]. The signs and symptoms of primary and secondary syphilis can be mild, and they might not be noticed. During the latent stage, there are no signs or symptoms. Tertiary syphilis is rare and develops in a subset of untreated syphilis infections; it can appear 10–30 years after infection was first acquired, and it can be fatal. Tertiary syphilis can affect multiple organ systems, including the brain, nerves, eyes, heart, blood vessels, liver, bones, and joints. Symptoms of tertiary syphilis vary depending on the organ system affected.

There are a worldwide estimated 10.6 million incident cases of syphilis per year[4] and high-income countries have seen a recent increase in syphilis rate[5,6]. In Europe in 2017, 33,189 cases of syphilis were reported in 28 countries, representing 7.1 cases per 100,000 inhabitants[6].

The diagnosis of syphilis relies primarily on the clinical presentation and, if deemed necessary, histopathologic analysis of cutaneous and mucosal tissues. These steps are followed up by direct pathogen detection in early pathogen-rich lesions or serologic examination for the presence of antibodies[7]. There are two types of antibody tests for the diagnosis of syphilis: nontreponemal and treponemal. Both types of tests are needed to confirm a diagnosis of syphilis.

Nontreponemal tests (e.g., VDRL and RPR) are not specific in targeting T. pallidum antigens[8], can produce false-positive results and, by themselves, are insufficient for diagnosis. Persons with a reactive nontreponemal test should always receive a treponemal test to confirm a syphilis diagnosis.

Treponemal tests (e.g. chemiluminescence immunoassays) detect antibodies that are specific for syphilis. Treponemal antibodies appear earlier than nontreponemal antibodies and usually remain detectable for life, even after successful treatment. Antitreponemal IgM antibodies are produced around two weeks after initial exposure and T. pallidum-specific IgG antibodies appear two weeks later[9]. After lesion onset in primary syphilis, both antitreponemal IgM and IgG antibodies may become detectable within 5-15 days[10]. Early targets of the humoral response are the carboxypeptidase 47-kDa lipoprotein TpN47 (Tp47) and flagellar proteins, followed by 15-kDa lipoprotein TpN15 (Tp15) and 17-kDa lipoprotein TpN17 (Tp17)[9]. In secondary syphilis, there is a relatively stronger increase in IgG3-specific antibodies. Treatment of syphilis results in a decrease in antitreponemal IgM antibodies which become undetectable within 6-12 months while IgG1 and IgG3 antibodies persist for years[9]. Elevated IgA antibodies have been observed in one third of patients with syphilis[9]. If a treponemal test is used for screening and the results are positive, a nontreponemal test with titer should be performed to confirm diagnosis and guide patient management decisions. Based on the results, further treponemal testing may be indicated.

Routine screening for syphilis is usually incorporated in genitourinary medical clinics (patients with sexually transmitted disease), in antenatal departments (pregnant women) and in blood transfusion services[10,11]. Other target groups are those with an increased risk of infection and those with a link with a country where syphilis has a high prevalence[10,11]. Patients with clinical signs and symptoms of syphilis should also be tested[10,11].

3. PRINCIPLE OF THE PROCEDURE

The method for determination of specific total antibodies to *Treponema pallidum* is a one-step sandwich chemiluminescence immunoassay (CLIA). Recombinant antigens specific for *Treponema pallidum* are used for coating magnetic particles (solid phase) and are linked to an isoluminol derivative (isoluminol-antigen conjugate). During the incubation, *Treponema pallidum* antibodies present in calibrators, samples or controls bind to the solid phase as well as to the antigen conjugate. After the 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-antigen conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of *Treponema pallidum* total antibody 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 *Treponema pallidum* recombinant antigens (obtained in *E. coli*) (approx. 50 µg/mL), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (1.4 mL)	CAL 1	Human serum/plasma containing low *Treponema pallidum* antibody levels (approx. 3.5 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 (1.4 mL)	CAL 2	Human serum/plasma containing high *Treponema pallidum* antibody levels (approx. 48 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 (13 mL)	DIL SPE	Proteins, EDTA, phosphate buffer, 0.2% ProClin™ 300, preservatives, an inert blue dye.
Conjugate (9 mL)	CONJ	*Treponema pallidum* recombinant antigens (obtained in *E. coli*) (approx. 1.4 µg/mL), conjugated to an isoluminol derivative, BSA, PBS buffer, 0.2% ProClin™ 300, preservatives.
Number of tests		200

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® Treponema Screen controls (negative and positive) (REF 310841).

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. PREPARATION OF 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.

An incomplete magnetic particles resuspension may cause variable and inaccurate analytical results.

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 Analyzer and LIAISON® XS Analyzer

– LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in 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 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.

8. STORAGE AND STABILITY OF REAGENT INTEGRAL

– **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

The correct type of specimen must be used with the assay. The followings have been tested and may be used:
– Serum;
– Plasma collected with the following anticoagulant:
.Sodium citrate;
.Lithium heparin;
.Sodium heparin;
.K2-EDTA.

Post- mortem specimens, collected up to 24 hours after death, have been tested and may be also used in the assay.

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

If samples are stored frozen, mix thawed samples well before testing. Further centrifugation of specimens removed from red cells, clot or gel separator (suggested between 3,000 and 10,000 g for 10 minutes) is recommended 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.

Cadaveric specimens should be stored following same indications than for living donors.

The minimum volume required for a single determination is 230 µL of specimen (80 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 four calibrations to be performed.

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

The analyzer should be calibrated in triplicate whenever one of the following conditions occurs:
– A new lot of reagent integral or of Starter Kit is used.
– The previous calibration was performed more than two (2) weeks before.
– The analyzer has been serviced.
– 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 Analyzer: Calibrator values are stored in the Radio Frequency IDentification transponder (RFID Tag).

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

11. ASSAY PROCEDURE

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

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 Analyzer 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. Dispense specimen diluent, coated magnetic particles and conjugate into the reaction module.
2. Dispense calibrators, controls or specimens.
3. Incubate.
4. Wash with Wash/System liquid.
5. Add the Starter reagents 1 and 2 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® Treponema Screen controls (REF 310841)
(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

The analyzer automatically calculates *Treponema pallidum* total antibody 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 – 70 Index.

The cut-off value discriminating between the presence and the absence of *Treponema pallidum* total antibodies has an index value of 1. Sample results should be interpreted as follows:
Samples with *Treponema pallidum* antibody levels below an index value of 0.9 should be graded non-reactive.
Samples with *Treponema pallidum* antibody levels ranging between an index value ≥0.9 and <1.1 should be graded *equivocal*.
Samples with *Treponema pallidum* antibody levels equal to or above an index value of 1.1 should be graded *initially reactive*.

A sample resulting *initially reactive* or *equivocal* at the first assay should be assayed in duplicate in order to confirm the initial result.

If a sample results repeatedly reactive in at least one replicate it should be considered reactive.

Samples which are non-reactive at the second test should be considered non-reactive.

A second sample should be collected and tested no less than one week later when the result is repeatedly equivocal.

A non-reactive result for total antibodies to *Treponema pallidum* generally indicates that the patient has not been infected, but does not always rule out acute syphilis, because the infection may be in its very early stage and the patient may be still unable to synthesize *Treponema pallidum* specific antibodies, or the antibodies may be present in undetectable levels. If clinical exposure to *Treponema pallidum* is suspected despite a non-reactive or equivocal finding, a second sample should be collected and tested later during the course of infection.

A reactive result for total antibodies to *Treponema pallidum* generally indicates exposure to the pathogen (acute or past infection). A single specimen, however, can only help estimate the serological status of the individual.

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.
– This test allows screening for the presence of *Treponema pallidum* total antibodies. It detects both recent and past infections, but it cannot distinguish between different antibody classes.
– Detection of *Treponema pallidum* total antibodies may indicate recent, past or successfully treated syphilis: this test, therefore, cannot discriminate between active and treated disease and, as a consequence, may not be used for determining response to therapy.
– In order to monitor the effectiveness of therapy clinical and serologic evaluation (non-treponemal tests) should be performed.
– LIAISON® Treponema Screen test may produce positive results that score negative with non-treponemal tests (VDRL, RPR), because it detects *Treponema pallidum* antibodies that persist often for life. RPR tests usually produce negative results in past infections, because they detect heterophilic antibodies that are present only in the early phase of infection.
– A non reactive test result does not exclude the possibility of exposure to or infection with syphilis. T. pallidum antibodies may be undetectable in some stages of the infection and in some clinical conditions.
– Test results are reported qualitatively as positive or negative for the presence of *Treponema pallidum* total antibodies. 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. Therefore a precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. Test results, however, must be interpreted with caution in immunocompromized individuals since their antibody levels may be affected by this condition.
– 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.
– Before testing cadaveric specimens, collection and centrifugation procedures should be carefully applied. After death, haemolysis and other changes (including proteolysis and dilution) occur in blood, which may lead to False Negative and False Positive in testing. In subjects transfused immediately prior to death high percentage of haemodilution can affect the performance of the test due to analyte dilution.

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, 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.

In addition, controlled studies of potentially exogenous substances showed no interference to each substance listed below in the LIAISON® Treponema Screen, up to the indicated concentration.

Tested Compound	Tested concentration
Benzathine penicillin G	110 mg/dL
Doxycicline	1.8 mg/dL
Ceftriaxone	84 mg/dL
Azythromycin	1.11 mg/dL
Interferon alpha 2a	6000 IE/mL
Interferon alpha 2b	6000 IE/mL
Interferon alpha 1b	6000 IE/mL
Entecavir	0.5 mg/L
Tenofovir	0.0978 mg/dL
Lamivudine	300 mg/L
Adefovir Dipivoxil	10 mg/L
Telbivudine	600 mg/L
Vitamin A	800 µg/dL
Vitamin B12	2850 pg/mL
Vitamin C	20 mg/dL
Vitamin D	450 ng/dL
Vitamin E	120 mg/L
Vitamin H (Biotin)	3510 ng/mL
Folic Acid	160 ng/mL
Acetaminophen	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Acetylsalicylic acid	3 mg/dL
Caffeine	10.8 mg/dL
Ethanol	600 mg/dL
Amiodarone	4.20 mg/dL
Atropine	0.06 mg/dL
Dopamine	0.0621 mg/dL
Epinephrine	0.25 mg/dL
Norepinephrine	0.0000507 mg/dL

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 hCMV, EBV, VZV, rubella virus, *Borrelia burgdorferi*, *Treponema denticola* or *Toxoplasma gondii* – *(b)* anti-nuclear (ANA) antibodies and rheumatoid factor (anti-Fc immunoglobulin) antibodies.

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 variability shown in the tables below did not result in sample misclassification. **The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.**

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

Repeatability	A	B	C	D
Number of determinations	20	20	20	20
Mean (index value)	0.13	8.17	51.48	58.53
Standard deviation	0.02	0.22	1.39	1.63
Coefficient of variation (%)	13.1	2.6	2.7	2.8

Reproducibility. Several replicates were performed in different days (one or two runs per day) with three different lots of integral to evaluate reproducibility. The tests were performed in two sites, in house and in an independent laboratory.

Reproducibility	SITE 1				SITE 2			
	E	F	G	H	E	F	G	H
LOT No. 01								
Number of determinations	20	20	20	20	11	11	11	11
Mean (index value)	0.18	11.02	40.20	56.45	0.15	12.50	41.68	57.47
Min value	0.14	9.40	26.90	47.00	0.10	11.30	38.10	42.40
Max value	0.20	12.80	46.70	61.00	0.19	14.00	47.00	66.70
Coefficient of variation (%)	10.8	7.3	12.1	5.9	15.4	7.0	7.1	11.2
LOT No. 02								
Number of determinations	20	20	20	20	11	11	11	11
Mean (index value)	0.13	12.24	38.08	56.20	0.07	13.15	40.00	57.36
Min value	0.09	11.00	33.20	49.00	0.05	11.90	36.70	50.70
Max value	0.26	13.90	44.30	62.30	0.09	14.00	43.00	64.00
Coefficient of variation (%)	39.2	6.3	8.5	5.9	21.9	4.6	7.9	6.3
LOT No. 03								
Number of determinations	20	20	20	20	11	11	11	11
Mean (index value)	0.06	12.60	40.83	54.45	0.03	12.57	38.85	55.80
Min value	0.04	11.40	34.70	47.30	0.03	11.10	33.70	49.70
Max value	0.10	13.50	49.30	61.30	0.03	13.60	42.30	62.00
Coefficient of variation (%)	24.7	3.7	12.1	8.4	0.0	5.7	9.1	6.6

Lot-to-Lot reproducibility was evaluated testing 5 samples in singleton, testing each sample in 6 runs on three product batches.

Reproducibility	LIAISON® Treponema Screen (Code 310840) on LIAISON® Analyzer				
Sample ID	I	L	M	N	O
Mean (Index value)	60.0	48.7	5.98	0.037	0.091
Inter-lot coefficient of variation (%)	3.6	5.8	4.8	10.4	15.9

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine 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. **The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.**

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

Repeatability	1	2	3	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.565	3.07	3.99	11.1
Standard deviation	0.051	0.056	0.082	0.34
Coefficient of variation (%)	9.1	1.8	2.0	3.0
Min. value	0.492	2.99	3.80	10.5
Max. value	0.656	3.19	4.12	11.6

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

Reproducibility	1	2	3	Positive control
Number of determinations	20	20	20	20
Mean (index value)	0.328	2.91	3.78	10.5
Standard deviation	0.053	0.10	0.12	0.47
Coefficient of variation (%)	16.0	3.6	3.2	4.5
Min. value	0.216	2.77	3.56	9.89
Max. value	0.422	3.17	4.06	11.2

15.4. Precision with LIAISON® XS Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine 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. **The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.**

Repeatability. Ninety (90) replicates were performed in the same test to evaluate repeatability. 6 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	4	5	6	7	8	9	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (index value)	0.884	1.411	8.281	30.47	50.02	59.59	1415	10.54
Standard deviation	0.024	0.038	0.135	0.760	0.856	1.456	32.64	0.280
Coefficient of variation (%)	2.8	2.7	1.6	2.5	1.7	2.6	2.3	2.7
Min. value	0.774	1.270	7.86	27.00	45.40	49.30	1261	9.00
Max. value	0.961	1.500	8.62	32.10	52.40	59.50	1540	11.3

*The precision of the negative control is evaluated in RLU because is out of the range.

Reproducibility. Ninety (90) replicates were performed in different days (one run per day) to evaluate reproducibility. 6 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	4	5	6	7	8	9	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (index value)	0.884	1.411	8.281	30.47	50.02	59.59	1415	10.54
Standard deviation	0.035	0.050	0.147	0.783	1.171	1.719	56.49	0.320
Coefficient of variation (%)	4.0	3.6	1.8	2.6	2.3	3.0	4.0	3.0
Min. value	0.774	1.270	7.86	27.00	45.40	49.30	1261	9.00
Max. value	0.961	1.500	8.62	32.10	52.40	59.50	1540	11.3

*The precision of the negative control is evaluated in RLU because is out of the range.

15.5. High-dose hook effect

Whenever samples containing extremely high antibody concentrations are tested in a one-step sandwich method, the hook effect can mimic concentrations lower than real.
Analysis of hook effect was evaluated by testing four high-titred samples positive for total antibodies to *Treponema pallidum*. All samples resulted in a concentration value above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.6. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were estimated by testing 7394 specimens from different populations, including Rapid Plasma Reagin (RPR) positive anti treponema negative specimens. The specimens were tested by several comparison methods and consensus between them as well as the available clinical and serological data were applied to define the expected results. Global results are summarized here below; detailed results follow.

Diagnostic specificity: 99.85% (6877/6887) - 95% confidence interval: 99.73-99.93%.

Diagnostic sensitivity: 99.80% (491/492) - 95% confidence interval: 98.86-99.96%.

BLOOD DONORS. Out of 5494 samples from non-selected blood donor population from three blood banks, 5486 results were negative, one result was equivocal and seven results were positive. Diagnostic specificity was 99.85% (5486/5494), 95% confidence interval: 99.71-99.94%.

HOSPITALIZED SAMPLES. Diagnostic specificity was assessed by testing 237 specimens from hospitalized samples. The specimens were tested by a CE reference kit. Three (3) specimens were graded reactive by the reference method and therefore were not included in data analysis.

233 non-reactive results were observed at screening in the expected negative population studied - diagnostic specificity: 99.57% (233/234) (95% confidence interval: 97.64-99.99%).

CLINICAL SAMPLES. A panel of 455 samples collected from different patients population were characterized by commercially available tests, such as RPR, TPHA, enzyme immunoassay and in-house Western blot, and were evaluated by LIAISON® Treponema Screen test.

Six results were equivocal and then excluded, because the comparison tests were not in agreement. Out of 449 classified positive samples, 449 results were positive by LIAISON® Treponema Screen test. Diagnostic sensitivity was 100% (449/449), calculated upon exclusion of equivocal results (95% confidence interval: 99.2-100%).

In an additional study, the ability of the LIAISON® Treponema Screen to detect anti-Treponema antibodies was evaluated by testing sequentially-collected specimens belonging to a seroconversion panel from donor who seroconverted over the course of the donation history. A commercially available, pre-characterized panel for Treponema antibodies was used, starting with a negative bleed and exhibiting narrow bleeding intervals.

The results show that the LIAISON® Treponema Screen test is aligned to commercially available assays.

CROSS-REACTIONS. 208 potentially cross-reactive samples were also tested: out of them, 54 results were positive for Borrelia burgdorferi sensu lato antibodies (24 Borrelia IgG positive characterized by immunoblot), 10 results were positive for EBV antibodies, 10 results were from systemic lupus erythematosus (SLE) patients, 15 results were positive for antiphospholipid antibodies, 9 results were positive for HIV antibodies, 10 results were positive for Streptococcus β-haemoliticus antibodies, 15 results were positive for Treponema denticola antibodies, 10 results were from multiparous pregnant women, 15 results were positive for HSV antibodies in pregnant women and 60 were positive for HSV antibodies. All those potentially cross-reactive samples were negative by LIAISON® Treponema Screen test.

PROSPECTIVE SAMPLES. 1000 samples were collected from non-selected laboratory routine specimens with relevant serological results (commercial enzyme immunoassay and in-house Western blot). As the comparison tests were not in agreement on five results, they were classified equivocal. Considering the results given by enzyme immunoassay and Western blot combined as consensus, diagnostic specificity was 99.89% (950/951 - 95% confidence interval: 99.42-100%) and diagnostic sensitivity was 97.67% (42/43 - 95% confidence interval: 87.71-99.94%), calculated upon exclusion of equivocal results.

15.7. Performance characteristics of cadaveric specimen testing

Performance characteristics of cadaveric specimens testing was determined by testing, according PEI validation protocol*, post-mortem specimens collected up to 24 hours after death in comparison to living donor specimens. 20 post-mortem samples were tested as unspiked and spiked at 2 levels: low positive and medium/high positive. The same procedure was performed with the same number of normal human serum from living donors, tested in parallel as reference to compare with post-mortem sample results. The results obtained were analyzed through calculation of percentage difference between mean of living donors results and mean of post-mortem results, at each reactivity level. In this study, the obtained percentage difference was equal or below 6,9% for each of the tested reactivity levels (see table below). Paired t-test analysis were performed between post-mortem and living donors specimens, spiked at low and medium/high positive levels, demonstrating not significantly difference on two groups (p value <0.05).

Repeteability was assessed using one post-mortem and one living donor specimens, spiked up to a low-level of reactivity with a human serum reactive for antibodies to *Treponema pallidum*. Each specimen was assessed in six replicates in the same run. The obtained percent coefficient of variation (CV%) did not exceed 15%. As reported in the table below 1.5% for the cadaveric specimen and 1.3% for the living donor were found in the study. The results refer to the group of investigated samples and are not guaranteed specifications, as differences may exist between laboratories and locations.

	Sample	Test results Means (index value)	Recovery (%) Post-mortem/Living donors	t-test p value	CV% 6 replicates
Neat	Post-Mortem unspiked	0.182	n.a.	n.a	n.a
	Living donors unspiked	<0.100			
Low Positive	Post-Mortem spiked	2.38	- 2.6	0.616	1.5
	Living donors spiked	2.45			1.3
Medium/high Positive	Post-Mortem spiked	5.89	7.2	0.104	n.a
	Living donors spiked	5.49			

* Paul Ehrlich Institute - Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, Anti-HCV-Assays, HBsAg and Anti-HBc Assays for Use with Cadaveric Samples - 08/05/2014

Summary of safety and performance is available on EUDAMED.

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

REFERENCES

1. Kubanov, A., Runina, A. & Deryabin, D. Novel Treponema pallidum Recombinant Antigens for Syphilis Diagnostics: Current Status and Future Prospects. Biomed Res Int 2017, 1436080, (2017).

2. Morshed, M. G. & Singh, A. E. Recent trends in the serologic diagnosis of syphilis. Clin Vaccine Immunol 22, 137-147, (2015).

3. Ho, E. L. & Lukehart, S. A. Syphilis: using modern approaches to understand an old disease. J Clin Invest 121, 4584-4592, (2011).

4. Tuddenham, S. & Ghanem, K. G. Emerging trends and persistent challenges in the management of adult syphilis. BMC Infect Dis 15, 351, (2015).

5. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2017. (U.S. Department of Health and Human Services, Atlanta, Georgia, United States, 2018).

6. European Centre for Disease Prevention and Control. Syphilis - Annual Epidemiological Report for 2017. (Stockholm, Sweden, 2018).

7. Schofer, H. et al. S2k guideline* "Diagnosis and therapy of syphilis"--short version. J Dtsch Dermatol Ges 13, 472-480, (2015).

8. Ahmed, N. & French, P. Interpretation of syphilis serology. Br J Hosp Med (Lond) 74, C104-107, (2013).

9. Sena, A. C., White, B. L. & Sparling, P. F. Novel Treponema pallidum serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 51, 700-708, (2010).

10. Ahmed, N. & French, P. Interpretation of syphilis serology. Br J Hosp Med (Lond) 74, C104-107, (2013).

11. Janier, M. et al. 2014 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol 28, 1581-1593, (2014).

12. Bowen et al., Clinical Biochemistry, 43, 4-25, 2010

200/007-893, 12 - 2025-10