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Changes: §15.5;
Deletions: -

LIAISON® Toxo IgG II (REF 310780)

1. INTENDED PURPOSE

The LIAISON® Toxo IgG II assay is a chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination of specific IgG antibodies to *Toxoplasma gondii* in human serum and plasma samples. The assay is intended for use as an aid in the diagnosis of *Toxoplasma gondii* infection and for screening of pregnant women.
The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Toxoplasmosis is caused by infection with the parasite Toxoplasma gondii. It is one of the most common parasitic infections in humans and is most typically asymptomatic. Primary infection in a pregnant woman can cause severe and disabling disease in the developing fetus.

Toxoplasma gondii is a protozoan parasite that infects most species of warm-blooded animals, including humans. Members of the cat family Felidae are the only known definitive hosts for the sexual stages of T. gondii and thus are the main reservoirs of infection.

WHO put in place strategies have emerged to decrease mother-to-child transmission through prompt identification of acutely infected pregnant women followed by appropriate treatment.

Toxoplasma gondii is prevalent worldwide. As reviewed in several publications, seropositivity rates show extreme geographic variations, ranging from less than 1% to greater than 95%. The highest overall rates are found in Latin American countries (mostly in Brazil), sub-Saharan Africa, the Middle East, and some parts of Asia. The lowest rates are reported in Southeast Asia. Estimates for North America are less than 25%, and those for Europe are mostly less than 36%.

Blood tests are required to establish whether or not the patient has pre-existing immunity or has acquired the infection post-conception and, if so, to establish when it occurred[2]. The detection of specific anti-Toxoplasma IgG and IgM antibodies are the most widely used.

IgG anti-Toxoplasma antibodies are detectable 2 to 4 weeks after infection and, depending on the individual responses of patients, they can also persist throughout the whole life. A positive test for IgG during pregnancy period indicates that the fetus is not at risk.

Anti-Toxoplasma IgM is traditionally tested in parallel to IgG to alert the physician to a possible acute infection when IgG are negative. When the IgM and the IgG are both positive the clinician have to know if it a recent or old (/chronic) infection. IgG avidity measures the strength of the antigen-antibody binding, which increases with the time elapsed since infection. IgG antibodies produced during the first month after primary infection are of low avidity, whereas those produced several months or a year past infection exhibit high avidity. A clinician based on test with high avidity can decide to not start any treatment, because it is chronic disease.

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgG to *Toxoplasma gondii* is an indirect chemiluminescence immunoassay (CLIA). *Toxoplasma gondii* is 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, *Toxoplasma gondii* antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with *Toxoplasma gondii* IgG 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 *Toxoplasma gondii* IgG concentration present in calibrators, samples or controls.

*(LIAISON® Analyzer, LIAISON® XL and LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥0.25% solid) coated with inactivated *Toxoplasma gondii* (RH strain) (minimum 113 µg/mL), obtained from sonicated and detergent-extracted trophozoites, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.7 mL)	CAL 1	Human serum/plasma containing low *Toxoplasma gondii* IgG levels (approx. 15 IU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (IU/mL) are referenced to the National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).
Calibrator 2 (2.7 mL)	CAL 2	Human serum/plasma containing high *Toxoplasma gondii* IgG levels (approx. 300 IU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (IU/mL) are referenced to the National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).
Specimen diluent (2 x 28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal antibodies to human IgG (minimum 10 ng/mL) conjugated to an isoluminol derivative, 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® Toxo IgG II controls (negative and positive) (REF 310781).

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 LIAISON®, LIAISON® XL and LIAISON® XS 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 labeled as follows:

REAGENTS:	CAL 1, CAL 2, DIL SPE, CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request.
For additional information see Safety Data Sheets available on www.diasorin.com.

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.

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.

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 specimen type must be used in the assay. Following matrices have been tested and may be used:
– serum,
– heparin plasma,
– K2-EDTA plasma,
– sodium 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:
– the 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.

The minimum volume required for a single determination is 170 µL of specimen (20 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:
– A new lot of reagent integral or of Starter Kit is used.
– The previous calibration was performed more than two (2) weeks before.
– 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 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 reagent integral Radio Frequency IDentification transponder (RFID Tag).
LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

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

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.

The analyzer operations are as follows:
1. Dispense diluent and coated magnetic particles into the reaction module.
2. Dispense calibrators, controls or specimens.
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® Toxo IgG II controls (REF 310781)
(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 lies 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 *Toxoplasma gondii* IgG antibody concentrations expressed as IU/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. 3 to 400 IU/mL *Toxoplasma gondii* 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 1:20). 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 18 sample predilutions to be performed.

Sample results should be interpreted as follows:
Samples with *Toxoplasma gondii* IgG concentrations below 7.2 IU/mL should be graded *negative*.
Samples with *Toxoplasma gondii* IgG concentrations ranging between 7.2 and 8.8 IU/mL should be graded *equivocal*.
Samples with *Toxoplasma gondii* IgG concentrations equal to or above 8.8 IU/mL should be graded *positive*.

A negative result indicates that immunity has not been acquired, but does not rule out acute infection. It should be underlined that the test usually scores negative in infected patients during the incubation period and the early stages of infection. If exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested no less than one or two weeks later. A positive result generally indicates either recent or past exposure to the pathogen. However, if IgG test scores positive in the presence of IgM antibodies, recent infection may be postulated. Serological data from detection of additional *Toxoplasma gondii* markers may provide useful information for clinical interpretation of results.Test results are reported quantitatively as positive or negative for the presence of *Toxoplasma gondii* 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.

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.

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, EDTA, heparin), haemolysis (up to 500 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® Toxo IgG II, up to indicated concentration.

Tested Compound	Tested concentration
Pyrimethamine	12.5 mg/L
Sulfadiazine	0.6 mg/L
Spiramycin	6 mg/L
Folic Acid	160 ng/mL
Acetaminophen	20 mg/dL
Ibuprofen	50 mg/dL
Acetylsalicylic acid	50 mg/dL

Cross-reactions. 105 potentially cross-reactive samples were tested, positive for IgM or IgG antibodies to one or more etiologic agent(s). 22 samples were positive for *Borrelia burgdorferi* IgM and/or IgG, 8 samples were positive for *Treponema pallidum* IgG, 7 samples were positive for hCMV IgM and/or IgG, 5 samples were positive for VZV IgG, 4 samples were positive for rubella virus IgG, 3 samples were positive for EBV VCA IgM, 32 samples were positive for anti-nuclear (ANA) antibodies, 13 were positive for human anti-mouse antibodies (HAMA) and 11 were positive for rheumatoid factor (anti-Fc immunoglobulin) antibodies. All potentially cross-reactive samples scored negative by LIAISON® Toxo IgG II test.

15.2. Precision with LIAISON® 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.

Repeatability	A	B	C	D	E	F
Number of determinations	20	20	20	20	20	20
Mean (IU/mL)	4.20	15.90	19.50	49.50	79.10	137.00
Standard deviation	0.10	0.24	0.75	1.54	4.48	6.72
Coefficient of variation (%)	2.5	1.5	3.9	3.1	5.7	4.9

Reproducibility	A	B	C	D	E	F
Number of determinations	20	20	20	20	20	20
Mean (IU/mL)	4.20	15.50	18.90	51.80	89.10	165.00
Standard deviation	0.27	1.54	1.12	4.63	10.68	18.52
Coefficient of variation (%)	6.4	9.9	5.9	8.9	12.0	11.2

Lot-to-Lot Reproducibility. Six (6) samples, containing different level of specific analyte, were assayed with two different batches in twenty-four (24) replicates (twelve (12) for each lot) with one (1) LIAISON® instrument in order to determine lot-to-lot reproducibility of the assay.

	LIAISON® Toxo IgG II (Code 310780) on LIAISON®					
Sample ID	A	B	C	D	E	F
Mean (IU/mL)	4.3	15.7	20.1	52.8	91.1	166.7
Inter-lot coefficient of variation (%)	10.1	8.0	7.4	7.5	7.1	10.8

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.

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 (IU/mL)	14.2	27.5	37.1	40.2	47.1	67.1	111	0.944	33.4
Standard deviation	0.70	1.41	1.16	1.35	2.53	4.13	5.36	0.042	1.40
Coefficient of variation (%)	5.0	5.1	3.1	3.4	5.4	6.2	4.8	4.5	4.2
Min. value	13.3	24.8	35.2	38.2	42.6	58.6	99.8	0.842	31.2
Max. value	16.2	29.3	38.9	43.2	52.4	76.8	120	1.00	35.5

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

Reproducibility	8	9	10	11	12	13	14	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (IU/mL)	8.65	19.5	30.9	43.7	57.1	100	186	0.245	29.2
Standard deviation	0.81	2.57	3.18	4.05	5.75	8.82	20.38	0.049	2.32
Coefficient of variation (%)	9.3	13.2	10.3	9.3	10.1	8.8	10.9	20.1	8.0
Min. value	7.50	12.5	25.3	35.4	46.3	85.5	163	0.0959	23.4
Max. value	10.7	25.1	37.9	50.4	65.7	122	227	0.321	33.2

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® Toxo IgG II Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 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 Toxo IgG II 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. 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	15	16	17	18	19	20	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (IU/mL)	6.48	8.53	18.5	30.3	36.7	104	13182*	34.2
Standard deviation	0.09	0.13	0.58	0.53	0.81	2.27	354	0.84
Coefficient of variation (%)	1.3	1.6	3.1	1.7	2.2	2.2	2.7	2.4
Min. value	5.82	7.60	15.3	27.0	31.9	85.8	10929	26.8
Max. value	7.12	9.54	20.6	33.9	41.5	123	15160	40.3

*Negative control kit precision is based on signal (RLU).

Reproducibility. Ninety 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	15	16	17	18	19	20	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (IU/mL)	6.48	8.53	18.5	30.3	36.7	104	13182*	34.2
Standard deviation	0.33	0.46	1.18	1.72	2.23	9.61	1015	2.94
Coefficient of variation (%)	5.0	5.4	6.4	5.7	6.1	9.2	7.7	8.6
Min. value	5.82	7.60	15.3	27.0	32.0	85.8	10929	26.8
Max. value	7.12	9.54	20.6	33.9	41.5	123	15160	40.3

*Negative control kit precision is based on signal (RLU).

15.5. Linearity and Trueness

The assay linearity has been checked by the dilution test.

Dilution test. Four serum samples containing high concentrations of *Toxoplasma gondii* IgG were tested as such and after serially diluting with the specimen diluent. *Toxoplasma gondii* IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.992 to 0.999.

Dilution	Expected concentration, IU/mL	Measured concentration, IU/mL	% Recovery	Dilution	Expected concentration, IU/mL	Measured concentration, IU/mL	% Recovery
neat	–	1124.0	–	neat	–	1384.0	–
1:8	140.5	135.0	96.1	1:8	173.0	161.0	93.1
1:16	70.3	83.3	118.5	1:16	86.5	81.5	94.2
1:32	35.1	43.4	123.6	1:32	43.3	40.6	93.8
1:64	17.6	22.7	128.9	1:64	21.6	19.2	88.9
1:128	8.8	10.2	115.9	1:128	10.8	8.4	77.8
1:256	4.4	4.1	93.2	1:256	5.4	3.4	62.9
neat	–	1568.0	–	neat	–	3264.0	–
1:16	98.0	110.0	112.2	1:32	102.0	104.0	101.9
1:32	49.0	48.8	99.6	1:64	51.0	53.5	104.9
1:64	24.5	24.4	99.6	1:128	25.5	23.9	93.7
1:128	12.3	12.0	98.0	1:256	12.8	11.1	86.7
1:256	6.1	5.0	81.6	1:512	6.4	4.6	71.9

The trueness evaluation was performed referred to National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).

Serial dilution in negative serum to cover the whole assay range of National Standard Serum for Toxoplasmosis E6 was tested in duplicate in recalibration mode with three assays on one LIAISON® instrument.

The results obtained are also elaborated through calculation of recovery % for each dilution point and calculation of correlation coefficient (R).

	Dilution	Measured IU/mL	Expected IU/mL	% Recovery	r^2 = 0.999
J	neat	>400	550.0	N.A.	r = 0.999
I	1:2	245.7	275.0	89.3	
H	1:4	120.9	137.5	88.0	
G	1:8	65.3	68.8	95.0	
F	1:16	38.2	34.4	111.2	
E	1:32	19.2	17.2	111.7	
D	1:64	10.0	8.6	116.8	
C	1:128	4.8	4.3	112.5	
B	1:256	2.3	2.2	107.1	
A	1:512	0.1	0.0	N.A.	

15.6. 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 was evaluated by testing six high-titred samples positive for *Toxoplasma gondii* IgG. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.7. Analytical and functional sensitivity

The Limit of Blank (LoB) for the LIAISON® Toxo IgG II is 1.72 IU/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® Toxo IgG II assay is 2.44 IU/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® Toxo IgG II assay is 2.63 IU/mL.

15.8. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 1000 specimens from different populations. 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. 58 specimens were unresolved either by the method under test or by the reference methods and therefore were not included in the data analysis.

Four positive and 702 negative results were observed in the expected negative population studied - diagnostic specificity: 99.43% (95% confidence interval: 98.56-99.84%).

No negative and 236 positive results were observed in the expected positive population studied - diagnostic sensitivity: 100% (95% confidence interval: 98.45-100%).

A 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.

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