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Changes: §1, §2, §4, §5, §6, §9, §12, §15.3, References;
Deletions: §14;

LIAISON® XL Toxo IgG Avidity (REF 310795)

1. INTENDED PURPOSE

The LIAISON® XL Toxo IgG Avidity assay uses indirect chemiluminescent immunoassay (CLIA) technology for the in vitro measurement of antigen-binding avidity of IgG antibodies to *Toxoplasma gondii* in human serum and plasma samples. The assay is intended as an aid in the diagnosis and staging the Toxoplasma infection, in subjects selected positive for the presence of Toxoplasma IgG and Toxoplasma IgM. 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, depending on the individual responses of patients will persist throughout 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 alerts 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 is 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 determination of antigen-binding avidity 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 to human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). The presence of strong bonds between IgG antibodies and *Toxoplasma gondii* (i.e., IgG avidity) in a given IgG-positive sample is detected by comparing the signal of a reference (i.e., non-treated) sample with the signal of the same sample after treating with urea, which dissociates weak bonds between IgG and *Toxoplasma gondii*. During the first incubation, *Toxoplasma gondii* antibodies present in calibrators, samples or controls bind to the solid phase (reference and treated samples). During the second incubation, the dissociating agent changes antigen-antibody bonds (treated sample only). Only high-avidity antibodies remain bound to the solid phase, whereas low-avidity antibodies are eliminated. During the final incubation, the antibody conjugate reacts with *Toxoplasma gondii* IgG already bound to the solid phase (reference and treated samples). 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. The IgG avidity index is given by the ratio of urea-treated specimens to reference specimens.

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

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (1.5 mL)	SORB	Magnetic particles (≥0.25% solid) coated with inactivated *Toxoplasma gondii* (RH strain) (approx. 0.1125 mg/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. 287 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (U/mL) are referenced to an in-house preparation.
Calibrator 2 (2.7 mL)	CAL 2	Human serum/plasma containing high *Toxoplasma gondii* IgG levels (approx. 6600 U/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (U/mL) are referenced to an in-house preparation.
Specimen diluent (28 mL)	DIL SP	BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye.
Buffer B (13 mL)	BUF B	Borate buffer, urea, 0.2% ProClin™ 300, preservatives, an inert blue dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives.
Number of tests		25

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® XL Toxo IgG Avidity controls (low- and high-avidity) (REF 310796).

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 of human origin used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle. The analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.

Do not use kits or components beyond the expiration date given on the label.

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

REAGENTS:	CAL 1, CAL 2 DIL SPE, CONJ	BUF B
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 Aquatic chronic 3 H412 Repr. 1B H360FD
SIGNAL WORD:	Warning	Danger
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark	  GHS07 Exclamation mark GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. H360FD May damage fertility. May damage the unborn child
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/ vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.	P261 Avoid breathing dust/fume/gas/mist/vapours/ spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P363 Wash contaminated clothing before reuse. P202 Do not handle until all safety precautions have been red and understood. P308+P313 If exposed or concerned: get medical advice/attention.
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).	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); Boric Acid.

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

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. Repeat as necessary until the magnetic particles are completely resuspended. After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

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, calibrator in particular (position two 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. Load the integral into the reagent area once the foam has dissipated.

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® XLand LIAISON® XS analyzers

- LIAISON® XL 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® XL Toxo IgG Avidity Control Set instructions for use section for proper preparation and handling instructions.

8. STORAGE AND STABILITY OF REAGENT INTEGRAL

Upon receipt, the reagent integral must be stored in an upright position to facilitate later resuspension of magnetic particles. When the reagent integral is stored sealed and kept upright, the reagents are stable at 2-8°C up to the expiry date. Do not freeze. The reagent integral should not be used past the expiry date indicated on the kit and reagent integral labels. After removing the seals, the reagent integral is stable for eight weeks refrigerated either at 2-8°C or on board the instrument.

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,
- sodium citrate,
- potassium EDTA,
- lithium heparin,
- sodium heparin.

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 6 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).

Only specimens positive for Toxoplasma gondii IgG by LIAISON® Toxo IgG II (REF 310780) may be tested for IgG avidity.

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 eight 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 local DiaSorin technical support or representative.

LIAISON® Analyzer: Calibrator values are stored in the reagent integral bar codes.

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

This test requires the following assay files: TGAv, TGNT and TG-T.

To test specimens use TGAv.

Never use TGNT or TG-T.

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.

Specimens must be tested with both protocols in order to obtain the final results (avidity index). The analyzer operations are as follows:

Protocol A

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

Protocol B

1. Dispense controls or specimens into the reaction module (calibration is not performed).
2. Dispense coated magnetic particles.
3. Dispense specimen diluent.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense buffer B.
7. Incubate.
8. Wash with Wash/System liquid.
9. Dispense conjugate into the reaction module.
10. Incubate.
11. Wash with Wash/System liquid.
12. Add the Starter reagents 1 and 2 and measure the light emitted.

12. QUALITY CONTROL

LIAISON® XL Toxo IgG Avidity controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® XL Toxo IgG Avidity controls (REF 310796)
(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,

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 the antigen-binding avidity index for specific IgG to *Toxoplasma gondii* (ratio of ureatreated specimens to reference specimens) and grades the results. For details, refer to the relevant analyzer operator's manual.

Results of specimens with Toxoplasma gondii IgG concentrations below 15 IU/mL by LIAISON® Toxo IgG II (REF 310780) *should be interpreted with care. Subsequent samples should be collected and tested with a two- to three-week delay in order to establish whether the antibody levels and the avidity index are falling or rising.*

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

Measuring range. 0.010 to 0.950 avidity index (Av) of *Toxoplasma gondii* IgG.

Samples containing *Toxoplasma gondii* IgG antibody levels above the measuring range (higher than 400 IU/mL) by LIAISON® Toxo IgG II (REF 310780) should be prediluted with specimen diluent by the Dilute function of the instrument before performing Toxo IgG Avidity test. The recommended dilution factor is 1:10. When the 1:10 diluted samples still score above the measuring range of the avidity test, Toxo IgG Avidity test should be repeated after prediluting the samples 1:20.

Samples containing *Toxoplasma gondii* IgG antibody levels within the measuring range may be tested directly. In case the samples show levels above the measuring range of Toxo IgG Avidity test (i.e., alert Invalid Combi Partner displayed), they should be prediluted 1:10 and tested again.

The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens and the avidity index will be calculated. The specimen diluent excess available in the reagent integral allows up to 25 sample predilutions to be performed.

Sample results should be interpreted as follows:

An avidity index value for *Toxoplasma gondii* IgG below 0.200 should be graded as *low avidity*.

An avidity index value for *Toxoplasma gondii* IgG ranging between 0.200 and 0.300 should be graded *moderate avidity*.

An avidity index value for *Toxoplasma gondii* IgG equal to or above 0.300 should be graded as *high avidity*.

Samples with avidity index greater than 0.950 should be retested. If the avidity index value is confirmed, it should be graded as *high avidity*.

Samples giving an Invalid Combi Partner Alert (for LIAISON® Analyzer platform) or a Failed avidity (for LIAISON® XL and LIAISON® XS platforms) with a result of TG-T < 1.5 U/mL (<< 1.500 U/mL for LIAISON® XL and LIAISON® XS platforms) for urea-treated specimen should be retested. If, after the retest, this result is confirmed, sample should be graded as *low avidity*.

A low avidity index value suggests the possibility of primary infection acquired less than four months before sample collection.

Warning - *A low avidity index, however, does not exclude past infection, as a proportion of infected persons may exhibit persistence of low-avidity IgG antibodies*.

A moderate avidity index value does not rule out the possibility of recent infection but may indicate past infection with unaccomplished maturity of IgG avidity. An avidity index below 0.300 provides no clear-cut indication for recent infection.

A high avidity index value may exclude that primary infection was acquired less than four months before sample collection.

Serological data from detection of additional Toxoplasma gondii markers may provide useful information for clinical interpretation of results.

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

Repeat testing of samples with avidity index values close to the discrimination threshold (i.e., 0.200 and 0.300 Av) may show some fluctuations in specimen classification, depending on precision of the system. This expected behaviour does not affect performance characteristics of the assay.

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

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, sodium and lithium 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.

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

Repeatability	A	B	C	D	E	F	G	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.081	0.168	0.209	0.455	0.472	0.595	0.606	0.091	0.593
Min value	0.068	0.153	0.190	0.401	0.428	0.528	0.566	0.080	0.557
Max value	0.094	0.186	0.233	0.539	0.503	0.670	0.646	0.111	0.636
Coefficient of variation (%)	9.6	5.8	5.5	9.3	4.1	6.1	4.1	6.9	3.5

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

Reproducibility - Laboratory 1	A	B	C	D	E	F	G	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.077	0.184	0.224	0.465	0.551	0.601	0.588	0.097	0.521
Min value	0.065	0.168	0.185	0.374	0.434	0.482	0.476	0.082	0.428
Max value	0.098	0.201	0.253	0.532	0.622	0.675	0.691	0.117	0.612
Coefficient of variation (%)	11.7	5.0	8.7	10.8	10.7	9.9	10.1	9.0	10.4

Reproducibility - Laboratory 2	A	B	C	D	E	F	G	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.068	0.160	0.195	0.436	0.478	0.561	0.569	0.088	0.472
Min value	0.056	0.140	0.177	0.365	0.411	0.490	0.499	0.072	0.399
Max value	0.094	0.214	0.233	0.552	0.587	0.667	0.700	0.110	0.581
Coefficient of variation (%)	16.3	12.0	8.0	11.7	11.3	8.6	10.6	13.1	12.6

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. 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	1	2	3	4	5	6	7	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.073	0.181	0.228	0.353	0.544	0.557	0.606	0.126	0.628
Min value	0.065	0.155	0.203	0.304	0.479	0.492	0.468	0.104	0.585
Max value	0.088	0.199	0.248	0.428	0.620	0.656	0.690	0.142	0.657
Coefficient of variation (%)	8.8	5.5	6.8	9.8	7.9	7.1	9.6	7.2	3.8

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

Reproducibility - Laboratory 1	1	2	3	4	5	6	7	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.077	0.173	0.221	0.381	0.549	0.580	0.623	0.099	0.576
Min value	0.060	0.140	0.148	0.314	0.479	0.453	0.444	0.076	0.486
Max value	0.110	0.206	0.271	0.472	0.633	0.695	0.745	0.121	0.683
Coefficient of variation (%)	17.5	11.6	12.3	12.7	7.7	10.8	10.7	11.5	9.4

Reproducibility - Laboratory 2	1	2	3	4	5	6	7	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.087	0.190	0.240	0.422	0.571	0.633	0.715	0.104	0.624
Min value	0.071	0.168	0.208	0.357	0.434	0.550	0.605	0.085	0.510
Max value	0.108	0.218	0.278	0.526	0.656	0.718	0.824	0.131	0.787
Coefficient of variation (%)	12.6	8.0	8.3	9.7	8.3	8.4	8.4	9.4	9.6

Lot to lot consistency. The lot to lot consistency was calculated by the reproducibility data. The tests were performed in two different laboratories on three different batches.

Reproducibility - Laboratory 1	1	2	3	4	5	6	7	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.079	0.177	0.222	0.390	0.517	0.572	0.628	0.103	0.571
Min value	0.075	0.171	0.218	0.374	0.500	0.559	0.621	0.097	0.558
Max value	0.085	0.188	0.227	0.416	0.549	0.580	0.638	0.112	0.579
Coefficient of variation (%)	6.9	5.4	2.2	5.8	5.3	2.0	1.5	8.2	2.0

Reproducibility - Laboratory 2	1	2	3	4	5	6	7	Control Low	Control High
Number of determinations	20	20	20	20	20	20	20	20	20
Mean avidity index	0.092	0.205	0.251	0.431	0.571	0.652	0.689	0.113	0.635
Min value	0.087	0.190	0.240	0.413	0.544	0.633	0.670	0.104	0.624
Max value	0.094	0.224	0.259	0.458	0.597	0.670	0.715	0.130	0.643
Coefficient of variation (%)	4.6	8.5	3.9	5.5	4.6	2.8	3.3	12.3	1.5

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® XL Toxo IgG Avidity Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.
A coded panel comprised of 6 frozen samples containing different concentration of analyte and kit controls was used for the study.
The LIAISON® XL Control Toxo IgG Avidity 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 avidity index, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

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

Repeatability	8	9	10	11	12	13	Control Low	Control High
Number of determinations	90	90	90	90	90	90	90	90
Mean avidity index	0.064	0.318	0.600	0.217	0.174	0.230	0.120	0.548
Standard deviation	0.004	0.016	0.021	0.010	0.007	0.021	0.008	0.026
Coefficient of variation (%)	5.5	5.1	3.5	4.8	4.1	9.0	6.4	4.8
Min. value)	0.051	0.271	0.546	0.175	0.151	0.079	0.105	0.496
Max. value	0.081	0.389	0.676	0.247	0.197	0.272	0.149	0.647

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

Reproducibility	8	9	10	11	12	13	Control Low	Control High
Number of determinations	90	90	90	90	90	90	90	90
Mean avidity index	0.064	0.318	0.600	0.217	0.174	0.230	0.120	0.548
Standard deviation	0.006	0.018	0.023	0.012	0.011	0.023	0.009	0.028
Coefficient of variation (%)	8.7	5.8	3.8	5.5	6.3	9.8	7.7	5.2
Min. value	0.051	0.271	0.546	0.175	0.151	0.079	0.105	0.496
Max. value	0.081	0.389	0.676	0.247	0.197	0.272	0.149	0.647

15.5. Trueness

The assay trueness has been checked by the dilution test.

Dilution test. Four high avidity serum samples and four low avidity serum samples containing *Toxoplasma gondii* IgG were tested as such and after serially diluting with the specimen diluent. Avidity index values for *Toxoplasma gondii* IgG measured before and after specimen dilution did not result in discrepant sample classification.

Avidity	Dilution	Measured avidity index	Avidity	Dilution	Measured avidity index
	neat	< 0.010		neat	0.603
	1:2	< 0.010		1:2	0.433
Low	1:4	< 0.010	High	1:4	0.471
	1:8	< 0.010		1:8	0.467
	1:16	< 0.010		1:16	0.508
	neat	0.071		neat	0.449
	1:2	0.099		1:2	0.382
Low	1:4	0.098	High	1:4	0.559
	1:8	0.112		1:8	0.561
	1:16	0.096		1:16	0.589
	neat	0.179		neat	0.486
	1:2	0.152		1:2	0.574
Low	1:4	0.142	High	1:4	0.636
	1:8	0.150		1:8	0.641
	1:16	0.124		1:16	0.611
	neat	0.169		neat	0.589
	1:2	0.180		1:2	0.618
Low	1:4	0.182	High	1:4	0.628
	1:8	0.164		1:8	0.587
	1:16	0.166		1:16	0.574

15.6. Diagnostic performance

261 specimens were tested from different selected populations positive for *Toxoplasma gondii* IgG (93 patients affected by primary *Toxoplasma gondii* infection, 48 subjects with past *Toxoplasma gondii* infection, 49 subjects with past infection and long-lasting *Toxoplasma gondii* IgM, 30 subjects with reactivated Toxoplasma infection, 41 follow-up from patients with previous primary Toxoplasma infection). Test results were defined after taking into account the results of the reference method and the agreement with the patient's clinical classification by the investigator. The patient's clinical classification and follow-up, when available, as well as the test of other *Toxoplasma gondii* markers were used to support the test outcome.

The overall diagnostic concordance with the reference method was 98.1% (95% confidence interval: 95.58 - 99.37%). Considering the 5 out of 261 discordant results with the reference method, 3 samples resulted high avidity with the LIAISON® XL Toxo IgG assay but consistent with clinical classification (2 past infections and 1 reactivated), while 2 samples classified as follow-up resulted respectively at high and at low avidity.

In addition samples whose clinical definition was possible were distinguished as primary infection presumably acquired in the four months prior to sample collection and past infection acquired more than four months from sample collection.

No high-avidity, 18 moderate-avidity and 75 low-avidity results were observed in the population studied, who presumably acquired the infection in the four months prior to sample collection. When assessed with respect to clinical condition, diagnostic concordance was 100% (95% confidence interval: 96.11-100%).

4 low-avidity, 7 moderate-avidity and 116 high-avidity results were observed in the population studied, who presumably acquired the infection more than four months prior to sample collection. When assessed with respect to clinical condition, diagnostic concordance was 91.34% (95% confidence interval: 85.03-95.60%).

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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