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

CE 0459

Changes: §1, §2, §4, §5, §6, §7, §8, §9, §10, §12, §15.4, §15.5, §15.7, References;
Deletions: -

LIAISON® Toxo IgM (REF 310710)

1. INTENDED PURPOSE

The LIAISON® Toxo IgM assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination of specific IgM 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.

Anti-Toxoplasma IgM is traditionally tested in parallel to IgG and is useful for two reasons. First, in a susceptible patient, IgM alerts the physician to a possible acute infection before IgG antibodies are detected because IgM antibodies are the first to appear in the context of acute infection (1 or 2 weeks before IgG). After increasing for 1 month, IgM antibodies persist for a period that varies between patients. The second advantage of testing for IgM is therefore that its absence when IgG antibodies are detected helps to exclude a recent infection.

The interpretation of a positive test result for IgM antibodies requires great caution for several reasons, mainly because IgM can remain positive for months and even years, especially with hypersensitive techniques. In a large study based on 446 women who acquired Toxoplasma infection during pregnancy, the persistence of IgM was longest when tested with ISAGA, which still yielded positive results in 27% of patients 2 years after infection, compared with 9% when using the indirect fluorescent antibody test. Positive IgM should therefore never be used alone as a sign of acute infection. The difficulty of interpreting positive IgM tests in the presence of IgG antibodies is even greater when the IgM tests used only provide qualitative results and cannot distinguish between low-to-moderate levels versus high levels that are more likely to indicate a recent infection.

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgM to *Toxoplasma gondii* is an antibody capture chemiluminescence immunoassay (CLIA). IgG to human IgM (mouse, monoclonal) is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody to *Toxoplasma gondii* major surface antigen (SAG1) is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, IgM antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with *Toxoplasma gondii* antigen previously added and the immune complex thus formed reacts with IgM 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* IgM 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 IgG to human IgM (mouse, monoclonal) (minimum 100 µg/mL), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (1.5 mL)	CAL 1	Human serum/plasma containing low *Toxoplasma gondii* IgM levels (approx. 7.5 AU/mL), BSA, PBS buffer, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (1.5 mL)	CAL 2	Human serum/plasma containing high *Toxoplasma gondii* IgM levels (approx. 50 AU/mL), BSA, PBS buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Antigen (2.3 mL)	Ag	Inactivated *Toxoplasma gondii* (RH strain) (approx. 75 µg/mL) obtained from ruptured and detergent-extracted trophozoites, PBS buffer, betaine, 0.2% ProClin® 300, preservatives.
Specimen diluent (28 mL)	DIL SPE	BSA, PBS buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (21 mL)	CONJ	Mouse monoclonal antibodies to *Toxoplasma gondii* major surface antigen (SAG1) (approx. 0.15 µg/mL) conjugated to an isoluminol derivative, non-specific IgG (mouse polyclonal), foetal calf serum, BSA, PBS buffer, 0.2% ProClin® 300, preservatives, an inert red dye.
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 IgM controls (negative and positive) (REF 310711).

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:	CAL1, CAL2, Ag, DILSPE, CONJ
CLASSIFICATION:	Skin sens. 1 H317
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction.
PRECAUTIONARY STATEMENTS:	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.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin® 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request.

For additional information see Safety Data Sheets available on www.diasorin.com.

7. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:
Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. 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. Incomplete magnetic particle 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 to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

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

LIAISON® XL and LIAISON® XS analyzers

– LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
– Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

CONTROLS

Refer to the LIAISON® Toxo IgM Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY

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

9. SPECIMEN COLLECTION AND PREPARATION

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

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.

Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.

Package and label specimens in compliance with applicable regulations covering the transport of clinical specimens and infectious substances.

Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.

Uncontrolled transport conditions (in terms of temperature and time) may cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore, not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010).

A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells or gel separator. The following storage conditions showed no significant differences:
– room temperature storage should be avoided;
– 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to 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 calibrator 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 Starter Kit is used.
– The previous calibration was performed more than four (4) weeks before.
– Each time a new lot of integral is used.
– Control values lie outside the expected ranges.
– **LIAISON® and LIAISON® XL analyzers**: the analyzer has been serviced.
– **LIAISON® XS Analyzer**: after a technical intervention, only if required by the service procedure, as communicated by 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

For LIAISON® XL Analyzer only: this test requires the following assay files: ToxoM, Tox-M6 and Tox-M10.
To test specimens use Tox-M6 or Tox-M10.
Never use ToxoM.

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 calibrators, controls or specimens, coated magnetic particles and specimen diluent into the reaction module.
2. Incubate.
3. Wash with Wash/System liquid.
4. Dispense antigen and conjugate into the reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. 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 IgM controls (REF 310711):
(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.
Warning: 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 *Toxoplasma gondii* IgM antibody concentrations expressed as AU/mL and grades the results. For details, refer to the analyzer operator's manual.
Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.
Assay range. 3 to 160 AU/mL *Toxoplasma gondii* IgM. It is generally accepted that IgM-capture assays may present sample-dependent dilution tests (K. Hansen et al., J. Clin. Microbiol., **29** (1) : 166-173, 1991). As a consequence, specimen results greater than the upper assay range should be considered and reported as above 160 AU/mL.
Sample results should be interpreted as follows:
Samples with *Toxoplasma gondii* IgM concentrations below 6 AU/mL should be graded *negative*.
Samples with *Toxoplasma gondii* IgM concentrations ranging between 6 and 8 AU/mL should be graded *equivocal*. Detection of additional *Toxoplasma gondii* markers may provide useful information for clinical interpretation of results.
Samples with *Toxoplasma gondii* IgM concentrations equal to or above 8 AU/mL should be graded *positive*.
Assay file for Toxo IgM determination on the LIAISON® XL Analyzer with the above interpretation of results is **Tox-M6**.
A positive result is indicative of the presence of IgM from either acute infection or past infection with persistent IgM levels. A negative result, however, does not always rule out acute toxoplasmosis. If clinical 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.
Test results are reported quantitatively as positive or negative for the presence of *Toxoplasma gondii* IgM. However, diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgement.
Note - The LIAISON® Toxo IgM kit is designed to measure the concentration of *Toxoplasma gondii* IgM with highest sensitivity, thus allowing very early detection of antibodies at the onset of acute infection. As *Toxoplasma* IgM may persist over time and samples containing low levels of long-lasting IgM score positive in the test, laboratories should adopt additional diagnostic procedures, like IgG avidity determination and/or different IgM assays, as useful tools to interpret IgM reactivity.
Laboratories may also find it useful to adopt a higher cut-off to increase diagnostic specificity of the LIAISON® Toxo IgM kit. *Toxoplasma* IgM antibodies sharply rise in the early stage of infection, while long-lasting IgM typically persist at lower levels before disappearing: the window of IgM positivity during the course of infection is then stretched towards the early phase. The risk of undetected acute infection samples remains negligible and further minimized by the practice of collecting a second sample one or two weeks later from negative patients.
In case this option is chosen, sample results should be interpreted as follows:
Samples with *Toxoplasma gondii* IgM concentrations below 10 AU/mL should be graded *negative*.
Samples with *Toxoplasma gondii* IgM concentrations equal to or above 10 AU/mL should be graded *positive*.
To adopt the option at 10.0 AU/mL on the LIAISON® XL Analyzer please use assay file **Tox-M10**.

Refer to the table below for details on results observed during performance evaluation.

Site	Number of samples	Cut-off value	Diagnostic specificity	Diagnostic sensitivity		
				Acute toxoplasmosis	Persistent Toxo IgM	Toxo IgM positive samples
Pavia, Italy	895	6-8 AU/mL	97.3%	–	–	99.6%
		10 AU/mL	99.1%	–	–	91.8%
Paris, France	347	6-8 AU/mL	94.0%	100%	95.0%	97.3%
		10 AU/mL	97.5%	100%	90.0%	94.6%
CDC, GA, U.S.	97	6-8 AU/mL	96.9%	100%	–	100%
		10 AU/mL	96.9%	100%	–	100%
Palo Alto, CA, U.S.	200	6-8 AU/mL	100%	100%	100%	100%
		10 AU/mL	100%	100%	94.0%	97.0%

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 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), or by freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® Toxo IgM assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to those of *Toxoplasma* infection (EBV, hCMV, rubella virus, parvovirus B19), from antibodies to other organisms that may cause infectious diseases (*Treponema pallidum*, *Borrelia burgdorferi*, HSV, VZV, measles virus, mumps virus, HIV, HCV) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, HAMA or human anti-mouse antibodies). Samples for these studies were pre-screened with another commercially available *Toxoplasma* IgM assay. If found negative for *Toxoplasma* IgM antibodies those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays.

Condition	Number of expected negative samples	LIAISON® positive or equivocal results
Parvovirus B19 IgM + IgG antibodies	14	0
Rubella virus IgM antibodies	15	0
hCMV IgM antibodies	17	0
EBV IgM antibodies	8	0
HCV antibodies	10	0
HIV antibodies	36	0
HSV-1/2 IgM + IgG antibodies	15	0
Borrelia burgdorferi IgM + IgG antibodies	7	0
Mumps virus IgM + IgG antibodies	5	0
Measles virus IgM antibodies	5	0
VZV IgM antibodies	11	0
Treponema pallidum IgM + IgG antibodies	5	0
Anti-nuclear autoantibodies (ANA/ENA)	12	0
Rheumatoid factor (anti-Fc immunoglobulin)	10	0
Human anti-mouse antibodies (HAMA)	14	0
Hypergammaglobulinaemia	20	0
Total	204	0

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

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

Repeatability	A	B	C	D	E	F
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	6.2	11.7	13.0	28.9	33.4	14.9
Standard deviation (AU/mL)	0.43	1.29	1.21	3.16	4.24	1.07
Coefficient of variation (%)	7.0	11.0	9.3	10.9	12.7	7.0
Min value (AU/mL)	4.9	8.2	10.3	20.6	23.9	12.1
Max value (AU/mL)	6.7	13.0	15.1	32.9	38.4	16.1

Reproducibility. Twenty 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 (site 1) and in an independent laboratory (site 2) using the same instruments.

Reproducibility - Site 1	A	B	C	D	E	F
LOT No. 01						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	6.1	11.0	15.2	30.4	45.8	15.5
Standard deviation (AU/mL)	0.50	1.41	1.77	3.80	5.52	2.20
Coefficient of variation (%)	8.2	12.9	11.6	12.5	12.1	14.5
Min value (AU/mL)	5.2	8.3	11.9	23.0	31.0	12.2
Max value (AU/mL)	6.8	14.0	18.8	36.4	53.3	22.5
LOT No. 02						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	7.9	12.7	20.9	36.9	44.2	20.7
Standard deviation (AU/mL)	0.38	1.42	1.53	4.23	4.61	2.40
Coefficient of variation (%)	4.8	11.1	7.3	11.4	10.4	11.7
Min value (AU/mL)	7.3	10.2	17.7	24.6	32.9	17.7
Max value (AU/mL)	8.6	15.5	23.1	44.5	51.2	27.6
LOT No. 03						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	6.0	11.0	13.1	29.2	45.9	12.7
Standard deviation (AU/mL)	0.52	1.23	0.92	3.65	2.95	1.80
Coefficient of variation (%)	8.7	11.1	7.0	12.5	6.4	14.0
Min value (AU/mL)	5.1	8.4	11.1	19.5	41.1	10.9
Max value (AU/mL)	6.9	12.5	15.2	35.3	51.8	19.4
Inter-lot coefficient of variation (%)	7.2	11.7	8.7	12.1	9.6	13.4

Reproducibility - Site 2	A	B	C	D	E	F
LOT No. 01						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	6.5	10.4	15.3	29.8	36.9	18.3
Standard deviation (AU/mL)	0.64	0.88	1.99	3.09	5.00	2.00
Coefficient of variation (%)	9.9	8.5	13.0	10.3	13.5	11.1
Min value (AU/mL)	5.4	9.0	12.1	24.9	27.4	14.2
Max value (AU/mL)	7.5	12.5	19.2	35.1	46.0	23.2
LOT No. 02						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	9.3	15.9	24.1	42.7	46.6	29.0
Standard deviation (AU/mL)	0.64	1.83	2.57	4.52	5.22	3.60
Coefficient of variation (%)	6.9	11.5	10.7	10.6	11.2	12.4
Min value (AU/mL)	8.6	13.5	19.1	35.8	37.6	23.1
Max value (AU/mL)	10.5	19.2	28.6	53.8	54.9	34.0
LOT No. 03						
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	6.7	11.7	11.8	26.7	27.0	14.6
Standard deviation (AU/mL)	0.46	1.11	0.84	3.29	3.47	1.70
Coefficient of variation (%)	6.9	9.5	7.2	12.3	12.9	11.9
Min value (AU/mL)	5.7	9.5	10.0	18.1	19.7	11.7
Max value (AU/mL)	7.9	14.0	13.1	30.8	32.8	18.4
Inter-lot coefficient of variation (%)	7.9	9.8	10.3	11.1	12.5	11.8

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 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 in-house repeatability.

Repeatability	PC	1	2	3	4	5
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	20.6	15.6	31.5	45.6	79.2	107
Standard deviation (AU/mL)	1.34	0.52	1.03	3.09	3.81	4.59
Coefficient of variation (%)	6.5	3.3	3.3	6.8	4.3	4.3
Min value (AU/mL)	18.8	14.9	29.6	40.3	73.4	102
Max value (AU/mL)	23.5	17.2	33.4	54.4	88.0	122

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

Reproducibility	PC	1	2	3	4	5
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	23.2	14.6	31.7	48.0	85.5	121
Standard deviation (AU/mL)	1.63	1.14	2.30	3.97	8.22	11.35
Coefficient of variation (%)	7.0	7.9	7.3	8.3	9.6	9.4
Min value (AU/mL)	20.0	13.0	29.0	40.6	65.1	107
Max value (AU/mL)	26.3	17.5	37.3	55.5	96.1	150

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 IgM Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 7 frozen samples was used for the study.

The samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels.

The coded panel was tested on three LIAISON® XS Analyzers, in six replicates in a single run per day, for 5 operative days.

The mean Index value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same test to evaluate repeatability. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 test per day, over 5 operating days, on 3 units and one reagent lot.

Repeatability	6	7	8	9	10	11	12	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (AU/mL)	4.00	6.47	9.59	10.8	40.2	43.0	79.5	31.3
Standard deviation	0.09	0.15	0.19	0.18	1.07	1.00	1.63	0.80
Coefficient of variation (%)	2.1	2.3	1.9	1.7	2.7	2.3	2.0	2.6
Min. value (AU/mL)	3.54	5.88	8.75	9.71	36.3	39.1	71.6	28.3
Max. value (AU/mL)	4.39	6.94	10.2	11.6	44.1	45.9	89.1	34.0

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Reproducibility	6	7	8	9	10	11	12	Positive control
Number of determinations	90	90	90	90	90	90	90	90
Mean (AU/mL)	4.00	6.47	9.59	10.8	40.2	43.0	79.5	31.3
Standard deviation	0.15	0.23	0.33	0.35	1.53	1.57	2.96	1.38
Coefficient of variation (%)	3.7	3.5	3.4	3.2	3.8	3.7	3.7	4.4
Min. value (AU/mL)	3.54	5.88	8.75	9.71	36.3	39.1	71.6	28.3
Max. value (AU/mL)	4.39	6.94	10.2	11.6	44.1	45.9	89.1	34.0

15.5. Trueness by recovery test

One set formed of one sample having high anti-Toxo IgM level and one sample having low anti-Toxo IgM level (samples X and Y) were mixed in 1:2, 1:1 and 2:1 ratios and assayed. Percent recoveries were determined from results of undiluted samples. Measured versus expected Toxo IgM concentrations were analyzed by linear regression.

The observed correlation coefficients (r) was 0.996.

Set 1	Expected concentration, µg/dL	Measured concentration, µg/dL	% Recovery
X neat	--	4.15	--
2:1	36.4	30.7	84.3
1:1	53.1	53.7	101.2
1:2	69.4	73.4	105.7
Y neat	--	102	--

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 two high-titred samples positive for *Toxoplasma gondii* IgM. All samples resulted in high concentration values as expected, indicating no sample misclassification.

15.7. Analytical and functional sensitivity

The Limit of Blank (LoB) for the LIAISON® Toxo IgM assay is 1.680 AU/mL. Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.654 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Toxo IgM assay is 2.120 AU/mL. Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® Toxo IgM assay is 2.156 AU/mL.

15.8. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were originally assessed by testing 789 specimens from different selected populations (subjects never infected by *Toxoplasma gondii*, pregnant women, subjects affected by autoimmune diseases, patients affected by other infectious diseases with similar symptomatology, haemodialyzed patients, transplant recipients, AIDS patients, patients affected by primary toxoplasmosis, subjects with past toxoplasmosis, patients with suspected recurrent toxoplasmosis, subjects with long-lasting *Toxoplasma gondii* IgM). 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. 51 specimens were unresolved by the reference methods and therefore were not included in the data analysis.

5 positive, 4 equivocal (reactive) and 586 negative results were observed in the expected negative population studied - diagnostic specificity: 98.49% (95% confidence interval: 97.15-99.31%).

No negative and 143 positive results were observed in the expected positive population studied - diagnostic sensitivity: 100% (95% confidence interval: 97.45-100%).

Selection of the higher cut-off causes results to be revised as follows:
3 positive and 592 negative results were observed in the expected negative population studied - diagnostic specificity: 99.50% (95% confidence interval: 98.53-99.89%).

14 negative and 129 positive results were observed in the expected positive population studied - diagnostic sensitivity: 90.21% (95% confidence interval: 84.12-94.55%).
In addition to the performance evaluation study, several studies were executed in specialized laboratories to validate the assay ability to correctly detect acute toxoplasmosis. 279 highly selected samples were tested, out of which 149 samples were obtained from subjects affected by acute toxoplasmosis and 130 samples were obtained from subjects with past toxoplasmosis with long-lasting IgM.

149 results above 10 AU/mL were observed in the population studied of acute toxoplasmosis patients - diagnostic sensitivity: 100% (95% confidence interval: 97.56-100%).

4 results below 6 AU/mL, 3 results ranging between 6 and 8 AU/mL, 4 results ranging between 8 and 10 AU/mL and 119 results above 10 AU/mL were observed in the population studied of subjects with long-lasting IgM - diagnostic sensitivity: 91.54% (95% confidence interval: 85.37-95.70%).

Further evaluation studies showed equal or better diagnostic performance.

Prospective study. 370 residual specimens prospectively collected from non-selected subjects that were sent to the laboratory for *Toxoplasma* testing were tested by LIAISON® Toxo IgM and by a *Toxoplasma* IgM immunoassay. LIAISON® Toxo IgM scored 358 negative, three equivocal and nine positive results and showed an agreement of 97.8% with the comparison method. The discordant results were solved by additional *Toxoplasma* IgM assays and IgG avidity test and consensus between them was applied to define the expected results. After solving the discrepancies, the agreement was 99.2%.

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.

REFERENCES

1 - James B. McAuley
Congenital Toxoplasmosis
Journal of Pediatric Infectious Diseases Society Vol.3 Suppl 1 pp. 530-535 2014

2 - Remington and Klein's
Infectious diseases of the fetus and newborn Infant
Eighth Edition Elsevier chapter 31 pag 949-1042

3 - Global incidence and Burden of Congenital Toxoplasmosis, by Region of the World Health Organization From Torgerson PR, Mastroiacovo P:
The global burden of congenital toxoplasmosis: a systematic review, Bull World Health Organ 91:501-508, 2013.

4 - Milewska-Bobula B, Lipka B, Golab E, Debski R, Marczyska M, Paul M, Panasiuk A, Seroczyska M, Mazela J, Dunin-Wsowicz D.
Recommended management of Toxoplasma gondii infection in pregnant women and their children.
Przegl Epidemiol. 2015;69(2):291-8, 403-10. English, Polish.

5 - Tomasoni LR, Meroni V, Bonfanti C, Bollani L, Lanzarini P, Frusca T, Castelli F.
Multidisciplinary approach to congenital Toxoplasma infection: an Italian nationwide survey.
New Microbiol. 2014 Jul;37(3):347-54. Epub 2014 Jul 1.

6 - Villena I, Ancelle T, Delmas C, Garcia P, Brezin AP, Thulliez P, Wallon M, King L, Goulet V; Toxosurv network and National Reference Centre for Toxoplasmosis
Congenital toxoplasmosis in France in 2007: first results from a national surveillance system.
Euro Surveill. 2010 Jun 24;15(25). pii: 19600.

7 - Wallon M, Peyron F.
Congenital Toxoplasmosis: A Plea for a Neglected Disease.
Pathogens. 2018 Feb 23;7(1). pii: E25. doi: 10.3390/pathogens7010025.

8 - Prusa AR, Kasper DC, Sawers L, Walter E, Hayde M, Stillwaggon E.
Congenital toxoplasmosis in Austria: Prenatal screening for prevention is cost-saving.
PLoS Negl Trop Dis. 2017 Jul 10;11(7):e0005648. doi: 10.1371/journal.pntd.0005648. eCollection 2017 Jul.

9 - Lange AE, Thyrian JR, Wetzka S, Flessa S, Hoffmann W, Zygmunt M, Fusch C, Lode HN, Heckmann M.
The impact of socioeconomic factors on the efficiency of voluntary toxoplasmosis screening during pregnancy: a population-based study.
BMC Pregnancy Childbirth. 2016 Jul 29;16(1):197. doi: 10.1186/s12884-016-0966-0.

10 - BOWEN et al.,
Impact of blood collection devices on clinical chemistry assay
Clinical Biochemistry, 43, 4-25, 2010.

11 - Clinical and Laboratory Standards Institute (CLSI) C15-A3, Vol.34, No.12,
User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition

200/007-831, 14 - 2024-03