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LIAISON® Toxo IgM II (REF 310715)

1. INTENDED USE

The LIAISON® Toxo IgM II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer family* for the qualitative determination of IgM antibodies to *Toxoplasma gondii* in human serum samples. It is intended for use as an aid in the presumptive diagnosis of acute or recent *Toxoplasma gondii* infection, including pregnant women. It is recommended that the LIAISON® Toxo IgM II assay be performed in conjunction with a *Toxoplasma gondii* IgG assay.

This assay has not been cleared/approved by the FDA for blood/plasma donor screening.

Caution: U.S. Federal Law restricts this device to sale by or on the order of a physician.

2. SUMMARY AND EXPLANATION OF THE TEST

Toxoplasmosis is a quite widespread infectious disease caused by an intracellular protozoan parasite, called *Toxoplasma gondii*. The disease, affecting both man and warm-blooded animals, can be transmitted by ingestion of food infected or contaminated by oocysts; direct contagion from domestic animals; or transplacental infection to newborn[1]. Transmission of Toxoplasma through organ transplantation has also been reported in the literature[2]. In the normal adult population, toxoplasmosis has a generally benign course, being largely asymptomatic; sometimes mildly symptomatic (headache, sore throat, asthenia); or in rare cases accompanied by lymphadenitis. The prevalence of positive serological tests increases with age, indicating past exposure[3].

Cell-mediated immunity is generally involved in protecting from parasite infection. As a consequence, a symptomatic course is generally more frequent in immunocompromised subjects such patients undergoing immunosuppressive therapy, or patients with acquired immunodeficiency syndrome[4,6].

If the infection occurs in pregnant women, toxoplasmosis can cause a threat to the fetus with possible spontaneous abortion, prematurity or stillbirth, as the pathogen can be transmitted to the fetus via the placenta. The fetus whose mother is exposed to Toxoplasma infection during the first trimester of pregnancy develops severe lesions to the central nervous system that generally lead to fetal demise. Toxoplasma infection acquired during the second trimester may cause hydro-cephalus, mental and psychomotor retardation, blindness and cerebral calcifications. Toxoplasma infection, however, is most common during the third trimester, causing retinochoroiditis and other ocular lesions, lesions to the central nervous system and latent asymptomatic infection which may eventually develop into full-blown disease[5]. Because of the diversity or absence of symptoms, the detection of Toxoplasma infection during pregnancy has to be based not on clinical findings, but on maternal serology[5].

The serological diagnosis of acute toxoplasmosis allows adequate treatment which reduces the risks of the disease both in immunocompromised patients and pregnant women[1,5].

Specific IgG antibodies to Toxoplasma, which appear subsequent to IgM antibodies, rise gradually and peak two to five months after the onset of infection. Therefore, the presence of IgG is useful in distinguishing subjects who have acquired the disease from those who have not. This is particularly important in order to identify susceptible women of child-bearing age[1].

Specific IgM antibodies to Toxoplasma develop two to four weeks after the onset of infection rapidly increasing and gradually decline thereafter, disappearing in three to nine months. The presence of IgM in the absence of IgG or in the presence of low IgG levels is generally indicative of acute toxoplasmosis[7].

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative 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® and LIAISON® XL)

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.5 mL)	SORB	Magnetic particles coated with IgG to human IgM (mouse monoclonal), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (1.5 mL)	CAL 1	Human serum/defibrinated plasma containing low *Toxoplasma gondii* IgM levels, 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/defibrinated plasma containing high *Toxoplasma gondii* IgM levels, BSA, PBS buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Assay Buffer 1 (2.3 mL)	BUF 1	Inactivated *Toxoplasma gondii* (RH strain) obtained from ruptured and detergent-extracted trophozoites, stabilizing agents, Betaine, 0.2% ProClin® 300, preservatives.
Specimen Diluent (28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (21 mL)	CONJ	Mouse monoclonal antibodies to *Toxoplasma gondii* major surface antigen (SAG1) conjugated to an isoluminol derivative, non-specific IgG (mouse polyclonal), fetal calf serum, BSA, PBS buffer, 0.2% ProClin® 300, preservatives, an inert red dye.
Number of Tests	100	

ProClin® is a registered trademark of Rohm and Haas Co.

All reagents are supplied ready to use. The order of the 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® Module (REF 319130).
LIAISON® XL Disposable Tips (REF X0015).	–
LIAISON® XL Starter Kit (REF 319200).	LIAISON® Starter Kit (REF 319102) or
	LIAISON® XL Starter Kit (REF 319200).
–	LIAISON® Light Check (REF 319101).
	LIAISON® Light Check 12 (REF 319150).
LIAISON® Wash/System Liquid (REF 319100).	LIAISON® Wash/System Liquid (REF 319100).
LIAISON® XL Waste Bags (REF X0025).	LIAISON® Waste Bags (REF 450003).
–	LIAISON® Cleaning Kit (REF 310990).

Additional required materials

LIAISON® Control Toxo IgM II (REF 310716).

5. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.

- For Professional Use Only.
- Do not mix reagents from different lots.
- The human blood source material used to produce the components provided in this kit derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 when tested by an FDA-approved method and found to be non-reactive for syphilis when tested by a serological test. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen in the CDCNIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 2007, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections (8, 10).
- Do not eat, drink, smoke or apply cosmetics during the assay.
- 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.

– Chemical reagents should be handled in accordance with Good Laboratory Practices.
– Do not mix reagents from different reagents packs (even for the same reagent).
– Previously frozen samples should be thoroughly mixed after thawing and prior to testing.
– Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard and Safety Information

– Reagents in this kit are classified in accordance with the US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and European Union EC Regulation 1272/2008 (CLP) (for additional information see Safety Data Sheet available on www.diasorin.com).

Hazardous reagents are classified and labelled as follow:

REAGENTS:	CAL 1, CAL 2, BUF 1, DIL SPE, 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 labelled as EUH210 safety data sheets available on request.

Reagents containing sodium azide

– Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to “Decontamination of Laboratory Sink Drains to Remove Azide Salts”, in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

– The LIAISON® Analyzer family should be cleaned and decontaminated on a routine basis. See the relevant Operator's Manual for the procedures.
– Strict adherence to the instructions are necessary to obtain reliable results.

6. PREPARATION OF REAGENTS and REAGENT INTEGRAL

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 color 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, to ensure there is no foaming present before using the Integral. If foam is present after resuspension of the magnetic particles, place the Integral on the instrument and allow the foam to dissipate. The Integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of Integral into the reagent area

LIAISON® Analyzer

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

LIAISON® XL Analyzer

– LIAISON® XL Analyzer is 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® Control Toxo IgM II instructions for use section for proper preparation and handling instructions.

7. STORAGE AND STABILITY

REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in the dark in an upright position to facilitate re-suspension of magnetic particles. Refer to the Reagent Integral Preparation for resuspension instructions. When the Reagent Integral is stored unopened and kept upright, the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral should not be used past the expiry date indicated on the kit and Reagent Integral labels. After opening and removing the seals, the Reagent Integral is stable for four weeks when stored in a refrigerator at 2-8°C or when stored on-board the analyzer. Always use the same analyzer for Reagent Integrals already opened. Use the storage rack provided with the analyzer for upright storage of the Reagent Integral. Undue exposure to light should be avoided.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum may be used. Blood should be collected aseptically by venipuncture, allowed to clot and the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. If the assay is performed within 7 days of sample collection, the samples may be stored at 2-8°C; otherwise they should be aliquoted and stored frozen (–20°C or below). If samples are stored frozen, mix thawed samples well before testing. Twelve samples with different reactivity were stored for seven days at 2-8°C and underwent four freeze-thaw cycles. The results showed no significant differences.
The minimum specimen volume required for a single determination is 170 µL [20 µL specimen for testing + 150 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

9. CALIBRATION

Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows 4 calibrations to be performed.

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

– With each new lot of reagent (Reagent Integral or Starter Reagents).
– The previous calibration was performed more than four (4) weeks before.
– The values of the recommended LIAISON® Control Toxo IgM II lie outside the expected ranges.
– After each servicing of the analyzer.

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

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

10. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the analyzer.

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL Analyzer. Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency IDentification transponder (RFID Tag). In the event the RFID Tag cannot be read by the analyzer, the reagent 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 patient 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 reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. Add the Starter Reagents and measure the light emitted.

11. QUALITY CONTROL

The LIAISON® Control Toxo IgM II (REF 310716) is recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance.

Quality control is recommended once per day of use, or in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. It is recommended the user refer to CLSI document C24-A3 and 42 CFR 493.1256(c) for guidance on appropriate quality control practices.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Quality control could be performed by running the LIAISON® Control Toxo IgM II or dedicated commercial controls:

– at least once per day of use,
– whenever the kit is calibrated,
– whenever a new lot of Starter Reagents is used,

Control values must lie within the expected ranges: whenever one of the 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.

12. INTERPRETATION OF RESULTS

The analyzer automatically calculates *Toxoplasma gondii* IgM antibody concentrations expressed in AU/mL and grades the results. Relative Light Units (RLU), AU/mL and the qualitative result (POS, Neg or Eqv) are provided on the Analyzer printout for each patient result. For details, refer to the analyzer operator's manual.

The cut-off was validated by testing 1031 samples (901 negative and 130 positive). The samples represented patients sent to the laboratory for *Toxoplasma gondii* IgM testing, pregnant women, and frozen repository samples from individuals with a positive *Toxoplasma gondii* IgM result. A cumulative frequency distribution (ROC) analysis was performed to determine the optimum cut-off.

The cut-off value discriminating between the presence and the absence of *Toxoplasma gondii* IgM was determined to have an AU/mL value of 10 AU/mL. An equivocal zone of 8.0 – 9.9 AU/mL was applied to the assay to account for normal measurement imprecision.

Calibrators and controls may give different RLU or dose results on LIAISON® and LIAISON® XL.

Assay range. 3.0 to 160 AU/mL *Toxoplasma gondii* IgM.

Index	Results	Interpretation
< 8.0 AU/mL	Negative	Absence of detectable *Toxoplasma gondii* IgM antibodies. A negative result does not always rule out acute toxoplasmosis, because the infection may be in its very early stage and the patient has not developed *Toxoplasma gondii* specific IgM. If exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested three weeks later.
≥ 8.0 AU/mL and < 10 AU/mL	Equivocal	The equivocal sample should be retested. If the result remains in this range after repeat testing, a second sample should be collected and tested three weeks later.
≥ 10.0 AU/mL	Positive	Possible presence of detectable *Toxoplasma gondii* IgM antibodies. A specimen with a positive result should be further tested for *Toxoplasma gondii*.

Note - *The magnitude of the measured result is not indicative of the amount of antibody present*.

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.

Recommendations for the interpretation of *Toxoplasma gondii* assay results*

Anti-*T. gondii* IgG Result	Anti-*T. gondii* IgM Result	Report/Interpretation for humans, except infants
Negative	Negative	No serological evidence of infection with *Toxoplasma*. If symptoms persist, obtain a new specimen three weeks later for testing.
Negative	Equivocal	Possible early acute infection or false-positive IgM reaction. Obtain a new specimen three weeks later for IgG and IgM testing. If results for the second specimen remain the same, the patient is probably not infected with *Toxoplasma*.
Negative	Positive	Possible recent infection or false-positive IgM result. Obtain a new specimen three weeks later for IgG and IgM testing. If results for the second specimen remain the same, the IgM reaction is probably a false positive.
Equivocal	Negative	Indeterminate: obtain a new specimen for testing or retest this specimen for IgG in a different assay.
Equivocal	Equivocal	Indeterminate: obtain a new specimen for both IgG and IgM testing.
Equivocal	Positive	Possible recent infection with *Toxoplasma*. Obtain a new specimen for IgG and IgM testing three weeks later. If results for the second specimen remain the same or if the IgG becomes positive, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Negative	Infected with *Toxoplasma* for usually more than six months.
Positive	Equivocal	Previously infected with *Toxoplasma*, but equivocal IgM results may be due to recent infection or false-positive IgM reaction. Obtain a second specimen three weeks later for testing. If results for the second specimen remain the same, both specimens should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	Positive	Possible recent infection. Send the specimen to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

* As stated in the CLSI document M36-A, Vol. 24 No. 6 (13).

13. LIMITATIONS OF THE PROCEDURE

– The LIAISON® Toxo IgM II assay is not, in and of itself, diagnostic and should be considered in conjunction with the patient's clinical presentation/history and other laboratory test results.
– Infections such as Epstein-Barr virus, Cytomegalovirus and different hepatitis viruses may cause symptoms similar to toxoplasmosis and must be excluded before confirmation of diagnosis.
– The magnitude of the measured result is not indicative of the amount of antibody present.
– Samples collected early in the course of the infection may not have detectable levels of specific IgM. A nonreactive IgM result may be due to delayed seroconversion and does not rule out current infection. If clinical exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested.
– Specific IgM Antibodies are usually detected in patients with recent primary infection, but they may be found in patients with reactivated infections, and they are sometimes found in patients with no other detectable evidence of recent infection.
– Do not rely on any single test result as the sole determinant in diagnosing recently acquired infection. If acute infection is suspected, a patient sample should be tested for the presence of Toxoplasma –specific IgG and IgM Antibodies. The results should be interpreted using the suggested algorithm for interpretation provided in the Interpretation of results section.
– The longevity of the detection of IgM antibodies post infection was not evaluated for this device.
– The performance was not evaluated in immunocompromised patients or infants.
– Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). Once an Integral has been introduced to a particular analyzer type, it must always be used on that Analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be conducted between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON® or LIAISON® XL).
– Single components of the reagent integral should not be removed from the integral.
– 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.

14. EXPECTED VALUES

Prevalence

The LIAISON® Toxo IgM II was tested with prospectively collected samples from US and European subjects sent to the laboratory for *Toxoplasma gondii* testing (n= 204 from the US and n=600 from Europe) and from pregnant women (n=201) to evaluate the assays performance in these populations. The US samples sent to the lab for *Toxoplasma gondii* testing were from 8 males (3.9%) and 196 females (96.1%). Ages ranged from 18 to 42 years with 147samples where age was unknown. Age and gender were unknown for the European subjects sent to the lab for *Toxoplasma gondii* testing. The samples from pregnant women ranged in age from 14 to 44 years. There were 70 samples from subjects in the 1st trimester, 50 samples from subjects in the 2nd trimester, and 81 samples from subjects in the 3rd trimester of pregnancy.

The prevalence may vary depending upon geographical location, age, gender, type of test employed, specimen collection and handling procedures as well as clinical history of the patient.

The distribution of results for observed *Toxoplasma gondii* IgM in these populations as determined by the LIAISON® Toxo IgM II assay is summarized in the tables below. Equivocal results were not used in the prevalence calculation.

Prospectively collected Samples from Subjects sent to the Laboratory for *Toxoplasma gondii* Testing (Collected in the U.S.)

		Gender			LIAISON® Toxo IgM II Results			% Prevalence
Total	Age	Male	Female	Unknown	Pos	Eqv	Neg	
0	<1	0	0	0	0	0	0	0.0%
0	1-10	0	0	0	0	0	0	0.0%
8	11-20	0	8	0	0	0	8	0.0%
22	21-30	0	22	0	0	0	22	0.0%
21	31-40	2	19	0	0	0	21	0.0%
6	41-50	0	6	0	0	0	6	0.0%
0	51-60	0	0	0	0	0	0	0.0%
0	61-70	0	0	0	0	0	0	0.0%
147	Unknown	6	141	10	0	0	147	0.0%

Prospectively collected samples from Subjects sent to the Laboratory for *Toxoplasma gondii* Testing (Collected in Europe)

			LIAISON® Toxo IgM II Results			% Prevalence
Total	Age	Gender	Pos	Eqv	Neg	
600	Unknown	Unknown	100	0	500	16.67%

Prospectively collected Samples from Pregnant Women (Collected in the U.S.)

		LIAISON® Toxo IgM II Results			% Prevalence
Age	N	Pos	Eqv	Neg	
11-20	59	3	0	56	5.08%
21-30	100	0	1	99	0
31-40	37	0	0	37	0
41-50	5	0	0	5	0
Trimester	N	Pos	Eqv	Neg	
1	70	1	0	69	1.40%
2	50	2	1	47	4.00%
3	81	0	0	81	0.00%

15. SPECIFIC PERFORMANCE CHARACTERISTICS

Comparative Testing

Prospective studies were performed to compare the performance of the LIAISON® Toxo IgM II IgM assay to an FDA-cleared predicate device. The study consisted of 804 samples from individuals who were sent to the laboratory for Toxoplasma IgM testing (204 samples from US subjects and 600 samples form European subjects) and 201 samples from pregnant women.

The 204 individuals from the US prospective population were 96.1% Female (n=196) ranging in age from 18 to 42 years with 141 female samples of unknown age, and 3.9% Male (n=8), 2 samples aged 31 and the remaining 6 with unknown age. Age and gender from the 600 European prospective population are unknown. The samples from pregnant women ranged in age from 14 to 44 years. There were 70 samples from subjects in the 1st trimester, 50 samples from subjects in the 2nd trimester, and 81 samples from subjects in the 3rd trimester of pregnancy.

US Prospective Samples

LIAISON® Toxo IgM II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	0	0	0	0
Equivocal	0	0	0	0
Negative	1	0	203	204
Total	1	0	203	204

	Percent Agreement	Exact 95% Confidence Interval
Negative 203/203	100.0%	98.2 – 100.0%
Positive 0/1	NA	NA

European Prospective Samples

LIAISON® Toxo IgM II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	93	5	2	100
Equivocal	0	0	0	0
Negative	1	0	499	500
Total	94	5	501	600

	Percent Agreement	Exact 95% Confidence Interval
Negative 499/506	98.9%	97.1 – 99.4%
Positive 93/94	98.9%	94.3 – 99.7%

Prospective Samples Pregnant Women

LIAISON® Toxo IgM II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	1	0	2	3
Equivocal	0	0	1	1
Negative	1	2	194	197
Total	2	2	197	201

	Percent Agreement	Exact 95% Confidence Interval
Negative 194/197	98.5%	94.1 – 99.1%
Positive 1/4	25.0%	4.6 – 70.0%

Retrospective study

The retrospective population consisted of 33 samples from individuals who had a positive Toxoplasma IgM result by the comparator assay. There were 93.9% Females (n=31) and 6.1% Males (n=2) ranging in age from 15 years to 47 years.

Toxoplasma IgM Positive Retrospective Population

LIAISON® Toxo IgM II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	33	0	0	33
Equivocal	0	0	0	0
Negative	0	0	0	0
Total	33	0	0	33

	Percent Agreement	Exact 95% Confidence Interval
Positive 33/33	100.0%	89.7 – 99.9%

CDC Panel Study

The CDC (Centers for Disease Control and Prevention) Toxoplasma 1998 Human Serum Panel was tested by the LIAISON® Toxo IgM II assay. The panel is comprised of 32 Toxoplasma IgM true positive samples, and 65 Toxoplasma IgM true negative samples. The results were submitted to the CDC for data analysis. The LIAISON® Toxo IgM II assay correctly detected 32/32 Toxoplasma IgM true positive samples (100% agreement) and 65/65 Toxoplasma IgM true negative samples (100% agreement).

Note: These results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply endorsement of the LIAISON® Toxo IgM II assay by the CDC.

Precision

Precision was assessed by measuring repeatability at one site using two kit controls and seven serum samples prepared to span the measuring range of the assay. Mean, standard deviation, and coefficient of variation (%CV) were calculated using multiple sources of variability that include within-run, within-day, between-day, and total variability. The following results were obtained from one site with one kit lot assayed in duplicate in two assays per day over 20 operating days.

Sample ID	Sample N	Mean AU/mL	Within-Run		Within-day		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control*	80	<3.0*	23.92*	3.7%*	9.78*	1.5%*	17.06*	2.6%*	30.96*	4.8%*
Positive Control	80	18.4	0.46	2.5%	0.46	2.5%	1.40	7.6%	1.54	8.4%
Toxo IgM-A*	80	<3.0*	28.18*	3.2%*	15.26*	1.7%*	36.26*	4.1%*	48.39*	5.5%*
Toxo IgM-B	80	4.9	0.10	2.1%	0.10	2.0%	0.21	4.3%	0.25	5.2%
Toxo IgM-C	80	15.9	0.53	3.3%	0.40	2.5%	1.07	6.8%	1.26	8.0%
Toxo IgM-D	80	36.1	1.13	3.1%	1.03	2.8%	3.66	10.1%	3.97	11.0%
Toxo IgM-E	80	54.6	1.48	2.7%	1.27	2.3%	5.53	10.1%	5.86	10.7%
Toxo IgM-F	80	86.8	2.16	2.5%	2.16	2.5%	8.21	9.4%	8.75	10.1%
Toxo IgM-G	80	121.0	4.27	3.5%	2.38	2.0%	11.55	9.5%	12.54	10.4%

* Dose and corresponding AUs were below the reading range of the assay.

Reproducibility

Reproducibility was assessed across all three testing sites using two kit controls and 7 serum samples prepared to span the measuring range of the assay. Mean, standard deviation, and coefficient of variation (%CV) were calculated using multiple sources of variability that include within-run, within-day, between-day, site to site and total variability. The following results were obtained from three sites with two kit lots assayed in duplicate in two assays per day over 20 operating days.

Sample ID	Sample N	Mean AU/mL	Within-Run		Within-day		Between-Day		Site to Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control*	480	<3.0*	55.66*	8.1%*	34.30*	5.0%*	48.37*	7.1%*	33.92*	5.0%*	93.67*	13.7%*
Positive Control	480	18.1	0.76	4.20%	0.34	1.90%	1.62	9.00%	0.61	3.40%	1.89	10.50%
Toxo IgM-A*	480	<3.0*	38.12*	4.0%*	27.82*	2.9%*	58.87*	6.2%*	50.16*	5.3%*	105.01*	11.0%*
Toxo IgM-B	480	4.7	0.14	3.00%	0.1	2.10%	0.36	7.50%	0.14	2.90%	0.42	9.00%
Toxo IgM-C	480	15.6	0.52	3.40%	0.35	2.30%	1.31	8.40%	0.55	3.60%	1.53	9.90%
Toxo IgM-D	480	34.2	1.39	4.10%	0.89	2.60%	3.43	10.00%	2.33	6.80%	4.41	12.90%
Toxo IgM-E	480	52.5	2.22	4.20%	1.73	3.30%	5.65	10.80%	3.46	6.60%	7.08	13.50%
Toxo IgM-F	480	84.6	3.51	4.10%	2.28	2.70%	8.05	9.50%	4.52	5.30%	10.04	11.90%
Toxo IgM-G	480	114.9	4.74	4.10%	3.64	3.20%	12.41	10.80%	6.47	5.60%	15.13	13.20%

* Dose and corresponding AUs were below the reading range of the assay.

Potentially Interfering Substances

Controlled studies of potentially interfering substances on 2 negative and 1 positive samples near the clinical decision point showed no interference at the concentration for each substance listed below in the LIAISON® Toxo IgM II assay. The testing was based on CLSI-EP07-A2.

Substance	Tested Concentration
Triglycerides	3000 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	20 mg/dL
Conjugated bilirubin	30 mg/dL
Albumin	6000 mg/dL
Cholesterol	510 mg/dL

Cross reactivity

The cross-reactivity study for the LIAISON® Toxo IgM II assay was designed to evaluate potential interference from other viruses that may cause symptoms similar to toxoplasmosis infection or from the presence of potentially cross-reactive antibodies or substances.

Cross-reactive organism	Number of samples tested	Reference Toxo IgM Result	LIAISON® Toxo IgM II		
			POS	EQV	NEG
IgM anti-HAV	8	Negative	0	0	8
IgM anti-VZV	5	Negative	0	0	5
IgM anti-CMV	14	Negative	0	0	14
IgM anti-EBV	12	Negative	0	0	12
IgM anti-HSV 1/2	10	Negative	0	0	10
IgM anti-Rubella	6	Negative	0	0	6
ANA IgG	10	Negative	0	0	10
anti-HIV	10	Negative	0	0	10
anti-HCV	10	Negative	0	0	10
anti-HBc	10	Negative	0	0	10
HAMA	10	Negative	0	0	10
Rheumatoid Factor	10	Negative	0	0	10
Total	115		0	0	115

None of the tested conditions returned results consistent with a conclusion of cross-reactivity

High Dose Hook Effect

Whenever samples containing extremely high analyte concentrations are tested, the high-dose hook effect can mimic concentrations lower than real. Analysis of high-dose hook effect was evaluated by testing three samples with *Toxoplasma gondii* IgM levels out-of-range >160 AU/mL. The sample resulted in a calculated concentration value above the measuring range, indicating no hook effect was observed and no sample misclassification.

16. REFERENCES

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15. Clinical and Laboratory Standards Institute (CLSI) EP7-A2, Vol.25, No.27 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

1. INTENDED USE

The DiaSorin LIAISON® Control Toxo IgM II (negative and positive) is intended for use as assayed quality control samples to monitor the performance of the DiaSorin LIAISON® Toxo IgM II assay on the LIAISON® Analyzer family*.

The performance characteristics of LIAISON® Control Toxo IgM II have not been established for any other assays or instrument platforms different from LIAISON® and LIAISON® XL.

LIAISON® Analyzer: The certificate of analysis gives specific information on the lot of controls, which should be manually entered in the analyzer sofware prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

LIAISON® XL Analyzer: The certificate of analysis bar codes give specific information on the lot of controls and should be read by the hand-held bar code scanner of the LIAISON® XL Analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

Caution: U.S. Federal Law restricts this device to sale by or on the order of a physician.

2. SUMMARY AND EXPLANATION OF THE TEST

The DiaSorin LIAISON® Control Toxo IgM II contains human serum-based samples. The controls will assist in the evaluation of proper performance of the LIAISON® Toxo IgM II assay when performed on the LIAISON® Analyzer family.

3. MATERIALS PROVIDED

The following materials are provided in the LIAISON® Control Toxo IgM II.

Negative Control (2 x 0.7 mL)	CONTROL -	Human serum/plasma non-reactive for *Toxoplasma gondii* IgM antibodies. 0.2% ProClin®.
Positive Control (2 x 0.7 mL)	CONTROL +	Human serum/plasma reactive for *Toxoplasma gondii* IgM antibodies. 0.2% ProClin®.

ProClin® is a registered trademark of Rohm and Haas Co.

The controls are supplied ready to use. The concentration range for each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs. Each laboratory is responsible for adopting different limits to meet individual requirements.

4. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Controls are not kit lot specific and may be interchanged among different kit lots.
- The human blood source material used to produce the components provided in this kit derives from donations found to be nonreactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 when tested by an FDA-approved method and found to be non-reactive for syphilis when tested by a serological test. 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.
- The controls are not calibrators and should not be used for assay calibration.
- All products containing human source material should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 2007, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections.

*(LIAISON® and LIAISON® XL)

5. SAFETY PRECAUTIONS

- Do not eat, drink, smoke or apply cosmetics during the assay.
- 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.
- Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard and Safety Information

– Reagents in this kit are classified in accordance with the US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and European Union EC Regulation 1272/2008 (CLP) (for additional information see Safety Data Sheet available on www.diasorin.com).

Hazardous reagents are classified and labelled as follow:

REAGENTS:	CONTROL -, CONTROL +
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).

6. STORAGE AND STABILITY

Store the control set in an upright position at 2-8°C upon receipt and prior to use. The controls must be stored in an upright position to prevent adherence of the solution to the vial cap. Do not freeze. The control set is stable until the expiration date on the vials when stored at 2-8°C. The controls should not be used past the expiration date indicated on the vial labels. Once opened, controls are stable for eight weeks when properly stored at 2-8°C between two successive uses. Avoid microbial contamination of controls. Indications of possible deterioration include the presence of particulate matter in the liquid or significant deviation from previous results.

The minimum specimen volume required is 420 µL (20 µL specimen + 400 µL dead volume). Each control solution allows 24 tests to be performed.

Allow controls to reach room temperature prior to use and mix thoroughly by gentle inversion. Return controls to the refrigerator immediately after each use.

7. QUALITY CONTROL

Quality control should be performed once per day of use, or according to guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI document, C24-A3, and 42 CFR 493.1256 for guidance on appropriate quality control practices.

LIAISON® Control Toxo IgM II positive and negative controls are intended to monitor for substantial reagent failure. Whenever controls lie outside the expected ranges provided on the certificate of analysis, calibration should be repeated and controls and samples retested. Do not report patient results until control results are within expected ranges.

Strict adherence to the instructions for use of the LIAISON® Toxo IgM II assay is necessary to obtain reliable results.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

8. ASSIGNED VALUES

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

The certificate of analysis bar codes give specific information on the lot of controls and should be read by the hand-held bar code scanner of the analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

9. PROCEDURE

Remove caps from the controls and place controls into sample rack type “C” with the barcode showing outward and slide rack into the patient sample area. Control identification is detected by the bar code label or may be manually programmed into the instrument. Follow the analyzer operator’s manual to start the run.

10. LIMITATIONS

Control values for assays other than the LIAISON® Toxo IgM II assay have not been established. If users wish to use this control material with other assays, it is their responsibility to establish appropriate ranges.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate reference ranges should be established for all quality control materials used. If control values obtained after successful calibration lie repeatedly outside the expect ranges, the test should be repeated using an unopened control vial.

SYMBOLS USED WITH IVD DEVICES

Consult instructions for use.

IVD

In vitro diagnostic.

LOT

Lot No.

Use by:

+ 8°C

+ 2°C

Temperature limitation.

Caution, consult accompanying documents.

REF

Catalogue number.

Manufacturer.

Σ XX

For XX tests

CONT	Kit contents
SORB	Magnetic particles
CONJ	Conjugate
BUF 1	Assay Buffer 1
DIL SPE	Specimen Diluent
CAL 1	Calibrator
CAL 2	Calibrator
CONTROL –	Negative control
CONTROL +	Positive control

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