DiaSorin S.p.A. - Via Crescentino snc - 13040 Saluggia (VC) - Italy
DiaSorin Inc. - Stillwater, MN 55082, USA
www.diasorin.com
Tel. +39.0161.4871

LIAISON® Toxo IgG II (REF 310705)

1. INTENDED USE

The LIAISON® Toxo IgG II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer family* for the qualitative determination of specific IgG antibodies to *Toxoplasma gondii* in human serum. The results of this assay can be used as an aid in the assessment of the patient's serological status to infection with *Toxoplasma gondii* and in the determination of immune status of individuals including pregnant women.

Caution: This assay has not been cleared/approved by the FDA for blood/plasma donor screening.
U.S. Federal Law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants.

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 blood transfusions or 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 as 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 hydrocephalus, 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 on maternal serology rather than on clinical findings.

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

Specific IgG antibodies to *Toxoplasma* 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 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 declining thereafter, generally 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 IgG antibodies to *Toxoplasma gondii* (anti-Toxo IgG) is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the analyzer. The principal components of the test are magnetic particles (solid phase) coated with *Toxoplasma gondii* and a conjugate of mouse monoclonal antibodies to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, *Toxoplasma gondii* antibodies present in diluted calibrators, samples or controls bind to the solid phase. During the second incubation, the monoclonal antibody conjugate reacts with anti-Toxo IgG that is already bound to the solid phase. After each incubation, 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 therefore, the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of anti-Toxo IgG in calibrators, samples or controls.

*(LIAISON® and LIAISON® XL)

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.5 mL)	SORB	Magnetic particles coated with inactivated *Toxoplasma gondii* (RH strain) obtained from sonicated and detergent-extracted trophozoites, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.7 mL)	CAL 1	Human serum/defibrinated plasma containing low *Toxoplasma gondii* IgG levels, BSA, PBS buffer, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations (IU/mL) are referenced to the National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).
Calibrator 2 (2.7 mL)	CAL 2	Human serum/plasma containing high *Toxoplasma gondii* IgG levels, BSA, PBS buffer, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (IU/mL) are referenced to the National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).
Specimen Diluent (2 x 28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin® 300, an inert yellow dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative, BSA, phosphate buffer, 0.2% ProClin® 300, preservatives.
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 IgG II (REF 310706).

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- 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, 9].
- 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 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, 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 precaution:

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 IgG II instructions for use section for proper preparation and handling instructions.

7. STORAGE AND STABILITY OF 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 eight 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

This assay can only test human serum samples. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested. Check for and remove air bubbles before assaying. Samples may be stored at 2-8°C for seven days; otherwise they should be dispensed in aliquots and stored deep-frozen (–20°C or below). If samples are stored frozen, mix thawed samples well before testing. Fourteen samples with different reactivity underwent five freeze-thaw cycles. The results showed no significant differences. Self-defrosting freezers are not recommended for sample storage. The minimum volume required is 170 µL per specimen (20 µL specimen + 150 µL dead volume).

9. CALIBRATION

Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Calibrator values are stored in the Radio frequency Identification transponder (RFID Tag) for calibration of the particular Reagent Integral lot. Each calibration solution allows 4 calibrations to be performed. The calibrator concentration (IU/mL) is referenced to National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980).

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 IgG II lie outside the expected ranges.
– After each servicing of the analyzer.

Refer to the Analyzer Operator's Manual or Analyzer Quick Guide for calibration instructions.

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 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 conjugate into the reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. Add the Starter Kit and measure the light emitted.

11. QUALITY CONTROL

The LIAISON® Control Toxo IgG II (REF 310706) is recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performancee.

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)[10] 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 IgG II sera 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* IgG antibody concentrations expressed in IU/mL and grades the results. Relative Light Units (RLU), IU/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 1006 samples (631 negative and 365 positive). The samples represented patients sent to the laboratory for *Toxoplasma gondii* testing and non-selected pregnant women. 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* IgG was determined to have a value greater than or equal to 8.8 IU/mL. An equivocal zone value greater than or equal to 7.2 IU/mL and less than 8.8 IU/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.

Warning – If the sample result displays "invalid RLU" and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample upon retest still displays "invalid RLU", call DiaSorin Technical Support.

Assay range. 3.00 to 400 IU/mL *Toxoplasma gondii* IgG.

Index	Results	Interpretation
< 7.2 IU/mL	Negative	Absence of detectable *Toxoplasma gondii* IgG antibodies. A negative result does not rule out acute infection. The test usually scores negative in infected patients during the incubation period and the early stages of infection. If exposure to *Toxoplasma gondii* is suspected despite a negative finding, a second sample should be collected and tested one or two weeks later.
≥ 7.2 - < 8.8 IU/mL	Equivocal	The equivocal sample should be repeat tested. In case the result remains in this range after repeat testing, a second sample should be collected and tested no less than one or two weeks later.
≥ 8.8 IU/mL	Positive	Presence of detectable *Toxoplasma gondii* IgG antibodies. A positive result generally indicates either recent or past exposure to the pathogen. If IgG test scores positive in the presence of IgM antibodies, recent infection may be postulated. If IgG test scored positive in the absence of IgM antibodies, past infection may be postulated.

Note - *The magnitude of the measured result is not indicative of the amount of antibody present. The concentrations of anti-Toxoplasma gondii IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity*.

Serological data from detection of additional *Toxoplasma gondii* markers may provide useful information for clinical interpretation of results (refer to CLSI document M36-A, Vol. 24, No. 6[11] for suggested testing scheme).

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 judgment.

13. LIMITATIONS OF THE PROCEDURE

1. The test should be performed on serum only. The use of whole blood or plasma specimens has not been established.
2. The use of icteric or lipemic sera, or sera exhibiting hemolysis or microbial growth should be avoided.
3. Do not heat-inactivate sera.
4. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
5. 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
6. The performance was not evaluated in immunocompromised patients.
7. 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).
8. Single components of the reagent integral should not be removed from the integral.
9. This kit must not be used after the expiration date printed on the package label.
10. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
11. Bacterial contamination or heat inactivation of the specimens may affect the test results.

14. EXPECTED VALUES

Prevalence

The LIAISON® Toxo IgG 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=202) 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 147 samples 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 71 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* IgG in these populations as determined by the LIAISON® Toxo IgG II assay is summarized in the tables below.

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

		Gender			LIAISON® Toxo IgG 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	1	0	21	4.5%
21	31-40	2	19	0	1	0	20	4.8%
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	0	19	0	128	12.9%
204	-	8	196	0	21	0	183	10.3%

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

			LIAISON® Toxo IgG II Results			% Prevalence
Total	Age	Gender	Pos	Eqv	Neg	
600	Unknown	Unknown	342	2	256	57.0%

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

		LIAISON® Toxo IgG II Results			% Prevalence
Age	N	Pos	Eqv	Neg	
11-20	59	6	0	53	10.2%
21-30	101	4	0	97	4.0%
31-40	37	2	0	35	5.4%
41-50	5	0	0	5	0.0%
Trimester	N	Pos	Eqv	Neg	
1	71	6	0	65	8.5%
2	50	4	0	46	8.0%
3	81	2	0	79	2.5%

15. SPECIFIC PERFORMANCE CHARACTERISTICS

Comparative Testing

Prospective studies were performed to compare the performance of the LIAISON® Toxo IgG II assay to an FDA-cleared predicate device. The study consisted of 804 samples from individuals who were sent to the laboratory for *Toxoplasma plamosis* testing (204 samples from US subjects and 600 samples form European subjects) and 202 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 71 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 IgG II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	21	0	0	21
Equivocal	0	0	0	0
Negative	0	0	183	183
Total	21	0	183	204

	Percent Agreement	Exact 95% Confidence Interval
Negative 183/183	100.0%	98.0 – 100.0%
Positive 21/21	100.0%	84.5 – 100.0%

European Prospective Samples

LIAISON® Toxo IgG II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	329	7	6	342
Equivocal	0	0	2	2
Negative	2	2	252	256
Total	331	9	260	600

	Percent Agreement	Exact 95% Confidence Interval
Negative 252/267	94.3%	90.9 – 96.6%
Positive 329/333	98.8%	96.6 – 99.5%

Prospective Samples Pregnant Women

LIAISON® Toxo IgG II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	12	0	0	12
Equivocal	0	0	0	0
Negative	1	1	188	190
Total	13	1	188	202

	Percent Agreement	Exact 95% Confidence Interval
Negative 188/188	100.0%	98.0 – 100.0%
Positive 12/14	85.7%	60.1 – 96.0%

Retrospective study

The retrospective population consisted of 42 samples from individuals who had a positive Toxoplasma IgG result by an FDA cleared Toxoplasma IgG assay. There were 95.2% females (n=40) and 4.8% males (n=2) ranging in age from 0 years to 47 years.

Toxoplasma IgG Positive Retrospective Population

LIAISON® Toxo IgG II	Comparator Assay			Total
	Positive	Equivocal	Negative	
Positive	42	0	0	42
Equivocal	0	0	0	0
Negative	0	0	0	0
Total	42	0	0	42

	Percent Agreement	Exact 95% Confidence Interval
Positive 42/42	100.0%	91.8 – 100.0%

CDC Panel Study

The CDC (Centers for Disease Control and Prevention) Toxoplasma 1998 Human Serum Panel was tested by the LIAISON® Toxo IgG II assay. The panel is comprised of 70 Toxoplasma IgG true positive samples, and 30 Toxoplasma IgG true negative samples. The results were submitted to the CDC for data analysis. The LIAISON® Toxo IgG II assay correctly detected 70/70 Toxoplasma IgG true positive samples (100% sensitivity) and 30/30 Toxoplasma IgG true negative samples (100% specificity).

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 IgG II assay by the CDC.

Precision/Reproducibility

Assay precision was evaluated according to CLSI EP5-A2[12]. Six serum samples containing concentrations of analyte prepared to span the range of the assay and kit controls (positive and negative) were assayed in duplicate in two runs per day over 20 operating days.

The following repeatability results were obtained from the samples tested internally at DiaSorin Inc. in one kit lot.

Repeatability

Sample ID	Sample N	Mean IU/mL	Within-Run		Within-Day		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control*	80	<3.0	90.36*	5.6%*	89.13*	5.5%*	155.58*	9.6%*	200.8*	12.4%*
Positive Control	80	22.8	1.19	5.2%	0.68	3.0%	0.48	2.1%	1.45	6.4%
Sample #1*	80	<3.0	151.7*	6.3%*	48.72*	2.0%*	262.18*	10.9%*	306.8*	12.8%*
Sample #2	80	7.5	0.53	7.2%	0.54	7.2%	0.00	0.0%	0.75	10.0%
Sample #3	80	15.8	0.85	5.4%	0.58	3.7%	0.41	2.6%	1.11	7.0%
Sample #4	80	13.2	0.76	5.8%	0.82	6.2%	0.14	1.1%	1.13	8.5%
Sample #5	80	27.0	1.13	4.2%	1.21	4.5%	0.70	2.6%	1.80	6.6%
Sample #6	80	76.9	4.35	5.7%	2.94	3.8%	3.16	4.1%	6.12	8.0%

* Dose and corresponding RLU's were below the reading range of the assay. Precision calculations are based on signal (RLU) for the two samples.

The following reproducibility results were obtained from the same six samples and kit controls (positive and negative) tested at three sites in two kit lots assayed in duplicate in two runs per day over 20 operating days.

Reproducibility

Sample ID	Sample N	Mean IU/mL	Within-Run		Within-Day		Between-Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control*	480	<3.00	100.82*	6.4%*	87.89*	5.5%*	147.49	9.3%*	208.12*	13.1%*	320.20*	20.2%*
Positive Control	480	22.8	1.4	6.1%	0.74	3.3%	1.6	7.0%	0.7	3.1%	2.69	11.8%
Sample #1*	480	<3.00	136.38*	5.5%*	94.22*	3.8%*	215.71*	8.7%*	375.14*	15.2%*	603.16*	24.4%*
Sample #2	480	7.4	0.48	6.5%	0.33	4.5%	0.42	5.8%	0.21	2.8%	0.88	12.0%
Sample #3	480	15.3	0.79	5.2%	0.61	4.0%	0.8	5.2%	0.38	2.5%	1.72	11.2%
Sample #4	480	13.6	0.68	5.0%	0.6	4.4%	0.89	6.6%	0.44	3.2%	1.43	10.5%
Sample #5	480	26.9	1.23	4.6%	1.12	4.2%	1.43	5.3%	0.92	3.4%	2.81	10.5%
Sample #6	480	77.3	3.92	5.1%	4.42	5.7%	5.95	7.7%	2.77	3.6%	9.44	12.2%

* Dose and corresponding RLUs were below the reading range of the assay. Precision calculations are based on signal (RLU) for the two samples.

Potentially Interfering Substances

Controlled studies of potentially interfering substances on 3 samples close to clinical decision points showed no interference at the concentration for each substance listed below in the LIAISON® Toxo IgG II assay. The testing was based on CLSI-EP07-A2[13].

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

Cross reactivity

The cross-reactivity study for the LIAISON® Toxo IgG 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 or condition	Number of samples tested	Reference Toxo IgG Result	LIAISON® Toxo IgG II		
			POS	EQV	NEG
Anti-HAV antibodies	5	Negative	0	0	5
Anti-HBc antibodies	5	Negative	0	0	5
Anti-VZV IgG antibodies	5	Negative	0	0	5
Anti-Rubella IgG antibodies	5	Negative	0	0	5
Anti-CMV IgG antibodies	10	Negative	0	0	10
Anti-EBV EBNA IgG antibodies	10	Negative	0	0	10
Anti-EBV VCA antibodies	10	Negative	0	0	10
Anti-HSV 1 IgG antibodies	10	Negative	0	0	10
Anti-HSV 2 IgG antibodies	5	Negative	0	0	5
Anti-ANA IgG antibodies	5	Negative	0	0	5
Anti-ds DNA IgG antibodies	2	Negative	0	0	2
Anti-Measles IgG antibodies	10	Negative	0	0	10
Anti-Mumps IgG antibodies	10	Negative	0	0	10
Treponema total antibodies	5	Negative	0	0	5
Anti-Parvo IgG antibodies	5	Negative	0	0	5
anti-HIV	5	Negative	0	0	5
anti-HCV	5	Negative	0	0	5
HAMA	10	Negative	0	0	10
Rheumatoid Factor	3	Negative	0	0	3
Total	125		0	0	125

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* IgG levels out-of-range >400 IU/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

1. J.S. REMINGTON, R. McLEOD, G. DESMONTS
   Toxoplasmosis. In: Infectious Diseases of the Fetus and Newborn Infant, J.S. Remington, J.O. Klein eds., W.B. Saunders Co. Publ., 4th edition, p. 140-267 (1995).
2. J.P. DUBEY, C.P. BEATTIE
   Toxoplasmosis of Animals and Man, CRC Press, Inc., Boca Raton, FL (1988).
3. J.G. MONTOYA, J.S. REMINGTON
   Toxoplasma gondii. In: Principles and Practice of Infectious Diseases, G.L. Mandell, J.E. Bennett, R. Dolin eds., Churchill Livingstone Publ., 5th edition, p. 2858-2888 (2000).
4. L.H. KASPER
   Toxoplasma infection and Toxoplasmosis, Harrison's Principles of Internal Medicine, 13th edition, J.D. Jeffers, S.D. Boynton eds., McGraw-Hill, p. 903-908 (1994).
5. M. WILSON, J.B. McAULEY
   Toxoplasma. In: Manual of Clinical Microbiology, P.R. Murray et al. eds., ASM Press, 7th edition, p. 1374-1382 (1999).
6. S.-Y. WONG, J.S. REMINGTON
   Biology of *Toxoplasma gondii* (editorial review). AIDS, 7 : 299-316 (1993).
7. P. THULLIEZ et al.
   Diagnosis of Toxoplasma Infection in the Pregnant Woman and Unborn Child: Current Problems, Scand. J. Inf. Dis., 18-22 (1992).
8. Biosafety in Microbiological and Biomedical Laboratories, Chosewood L.C., Wilson D.E. (eds). Atlanta, GA, U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institutes of Health, 5th ed., Feb. 2007.
9. Clinical Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline - Third Edition. CLSI Document M29-A3. Villanova, PA; CLSI: 2005.
10. Clinical and Laboratory Standards Institute (CLSI) C24-A3, Vol.26, No.25, Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline - Third Edition.
11. Clinical Laboratory Standards Institute. Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii*; Approved Guideline, CLSI Document M36-A, Vol.24, No.6.
12. Clinical and Laboratory Standards Institute (CLSI) EP5-A2, Vol.24, No.25, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition.
13. Clinical and Laboratory Standards Institute (CLSI) EP7-A2, Vol.25, No.27 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

LIAISON® Control Toxo IgG II (REF 310706)

1. INTENDED USE

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

The performance characteristics of LIAISON® Control Toxo IgG II have not been established for any other *T. gondii* assay 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 IgG II contains human serum-based samples. The controls will assist in the evaluation of proper performance of the LIAISON® Toxo IgG II assay when performed on the LIAISON® Analyzer family.

3. MATERIALS PROVIDED

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

Negative Control (2 x 0.7 mL)	CONTROL -	Human serum or defibrinated plasma non-reactive for *T. gondii* antibodies and 0.2% ProClin® 300.
Positive Control (2 x 0.7 mL)	CONTROL +	Human serum or defibrinated plasma reactive for *T. gondii* antibodies and 0.2% ProClin® 300.

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.

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.

*(LIAISON® and LIAISON® XL)

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.

At the time of use, equilibrate controls to room temperature (20-25°C) before opening the vials and keep them on board the instrument only for the amount of time required for quality control testing. After use, stopper the vials promptly and store them at 2-8°C in an upright position.

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-A, and 42 CFR 493.1256 for guidance on appropriate quality control practices.

LIAISON® Control Toxo IgG 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 IgG 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 IgG 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

CAL	1

Calibrator

CAL	2

Calibrator

DIL	SPE

Specimen Diluent

CONTROL	–

Negative control

CONTROL	+

Positive control

For Customer Service in the U.S. and Canada call toll free: 1-800-328-1482.
200/008-927, 01 - 2013-12