

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

K131441

B. Purpose for Submission:

To obtain substantial equivalence for the DiaSorin LIAISON[®] IgM II and LIAISON[®] Control IgM II

C. Measurand:

Toxoplasma gondii IgM antibodies in human serum

D. Type of Test:

Chemiluminescence Immunoassay

E. Applicant:

DiaSorin Inc.

F. Proprietary and Established Names:

DiaSorin LIAISON[®] IgM II and LIAISON[®] Control IgM II

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3780 - *Toxoplasma gondii* Serological Reagents

2. Classification:

Class II

3. Product code:

LGD; Enzyme Linked Immunosorbent Assay, *Toxoplasma gondii*

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

LIAISON® Toxo IgM II

The DiaSorin LIAISON® Toxo IgM II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON XL Analyzer® for the qualitative determination of IgM antibodies to *Toxoplasma gondii* in human serum. The LIAISON® Toxo IgM II 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.

LIAISON® Control Toxo IgM II

The DiaSorin LIAISON® Control Toxo IgM II is intended for use as assayed quality control samples to monitor the performance of the DiaSorin LIAISON® Toxo IgM II assay on the LIAISON® XL Analyzer.

2. Indication(s) for use:

LIAISON® Toxo IgM II

The DiaSorin LIAISON® Toxo IgM II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON XL Analyzer® for the qualitative determination of IgM antibodies to *Toxoplasma gondii* in human serum. The LIAISON® Toxo IgM II 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.

LIAISON® Control Toxo IgM II

The DiaSorin LIAISON® Control Toxo IgM II is intended for use as assayed quality control samples to monitor the performance of the DiaSorin LIAISON® Toxo IgM II assay on the LIAISON® XL Analyzer.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

LIAISON[®] XL Analyzer

I. Device Description:

The method for qualitative determination of specific IgM to *Toxoplasma gondii* is an antibody capture chemiluminescence immunoassay (CLIA). The principal components of the test are magnetic particles (solid phase) coated with IgG (mouse, monoclonal) is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody to human IgM, *Toxoplasma gondii* antigen, and a conjugate of mouse monoclonal antibodies to *Toxoplasma gondii* 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 mouse monoclonal antibody conjugate reacts with *Toxoplasma gondii* antigen and the immune complex thus formed reacts with IgM already bound to the solid phase. After the incubations, 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.

All assay steps and incubations are performed by the LIAISON[®] XL Analyzer.

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 <i>Toxoplasma gondii</i> 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 <i>Toxoplasma gondii</i> 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 <i>Toxoplasma gondii</i> (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 <i>Toxoplasma gondii</i> 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 the containers in the reagent integral.

Materials required but not provided

LIAISON[®] XL Cuvettes ([REF] X0016)
LIAISON[®] XL Disposable Tips ([REF] X0015)
LIAISON[®] XL Starter Kit ([REF] 319200)
LIAISON[®] Wash/System Liquid ([REF] 319100)
LIAISON[®] XL Waste Bags ([REF] X0025)

Additional required materials

LIAISON[®] Control Toxo IgM II ([REF] 310716)

J. Substantial Equivalence Information:

1. Predicate device name(s):

Diamedix Is-Toxoplasma IgM Capture Test System

2. Predicate 510(k) number(s):

K001707

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The LIAISON® Toxo IgM II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of IgM antibodies to <i>Toxoplasma gondii</i> in human serum specimens. It is intended for use as an aid in the presumptive diagnosis of acute or recent <i>Toxoplasma gondii</i> infection, including pregnant women. It is recommended that the LIAISON® Toxo IgM II assay be performed in conjunction with a <i>Toxoplasma gondii</i> IgG assay. This assay has not been cleared/approved by the FDA for blood/plasma donor screening.	The Diamedix Is Toxoplasma IgM Capture Test Kit is a capture enzyme immunoassay (EIA) for the presumptive qualitative detection of IgM antibodies to <i>Toxoplasma gondii</i> in human serum by capture enzyme immunoassay. When performed in conjunction with an anti- <i>Toxoplasma gondii</i> IgG assay, the Is-Toxoplasma IgM Capture assay can be used as an aid in the presumptive diagnosis of acute, recent or reactivated <i>Toxoplasma gondii</i> infection. Performance has not been established in newborns. This product has not been cleared/approved by the FDA for blood/plasma donor screening.
Measured Analyte	IgM antibodies to <i>Toxoplasma gondii</i>	IgM antibodies to <i>Toxoplasma gondii</i>
Reagent Storage	On-board or in refrigerator @ 2-8°C	In refrigerator @ 2-8°C
Calibrators	Included in kit	Included in kit
Controls	2 levels (negative and positive)	2 levels (negative and positive)
Sample Matrix	Human serum	Human Serum
Antigen	<i>Toxoplasma gondii</i> , RH Strain	<i>Toxoplasma gondii</i> , RH Strain
Capture Antibody	Mouse monoclonal anti-human IgM	Mouse monoclonal anti-human IgM

Differences		
Item	Device	Predicate
Assay Type	Chemiluminescent Immunoassay	Enzyme Assay
Calibration	Two point verification of stored master curve	Single point cut-off calibrator
Unit of Measure	AU/mL	Index Value
Cut-Off	10.0 AU/mL	1.10 Index Value
Equivocal Zone	8.0 – 9.9 AU/mL	0.90 – 1.09 Index Value
Sample Size	20 µL	2 µL
Sample Handling/Processing	Automated	Manual or Automated
Assay Time	40 minutes	140 minutes
Controls	Provided separately	Included with kit
Capture Reagent	Magnetic particles coated with IgG to human IgM (mouse monoclonal)	Microwells coated with mouse monoclonal anti-human IgM (heavy chain)

Differences		
Item	Device	Predicate
Conjugate	Mouse monoclonal antibodies to <i>Toxoplasma gondii</i> conjugated to an isoluminol derivative	Mouse monoclonal anti- <i>Toxoplasma gondii</i> conjugated to horseradish peroxidase
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA microtiter plate reader)

AU = Arbitrary Units

K. Standard/Guidance Document Referenced (if applicable):

- EP05-A2, Evaluation of Precision Performance of Quality Measurement Methods; Approved Guideline - Second Edition 2004
- EP07-A2, Interference Testing in Clinical Chemistry – Approved Guideline – Second Edition 2005

L. Test Principle:

The test is a Chemiluminescence Immunoassay (Immunoassay technology based on the emission of light as a result of a chemical reaction). During the first incubation, IgM antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the mouse monoclonal 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 of the incubations, the unbound material was 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 the presence of *Toxoplasma gondii* IgM in calibrators, samples or controls.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

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

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	4.2%	0.34	1.9%	1.62	9.0%	0.61	3.4%	1.89	10.5%
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	3.0%	0.10	2.1%	0.36	7.5%	0.14	2.9%	0.42	9.0%
Toxo IgM-C	480	15.6	0.52	3.4%	0.35	2.3%	1.31	8.4%	0.55	3.6%	1.53	9.9%
Toxo IgM-D	480	34.2	1.39	4.1%	0.89	2.6%	3.43	10.0%	2.33	6.8%	4.41	12.9%
Toxo IgM-E	480	52.5	2.22	4.2%	1.73	3.3%	5.65	10.8%	3.46	6.6%	7.08	13.5%
Toxo IgM-F	480	84.6	3.51	4.1%	2.28	2.7%	8.05	9.5%	4.52	5.3%	10.04	11.9%
Toxo IgM-G	480	114.9	4.74	4.1%	3.64	3.2%	12.41	10.8%	6.47	5.6%	15.13	13.2%

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

b. Linearity/assay reportable range:

Not applicable – This assay is qualitative.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:

There is no international standard available for measuring anti-Toxoplasma IgM in serum; therefore, calibrators are traceable to an in-house reference preparation. Positive human serum/defibrinated plasma is serially diluted with negative serum/defibrinated plasma and tested in several assays and assigned Arbitrary Units (AU/mL) values spanning the range of the assay. Aliquots are stored frozen and used during the manufacturing of the kit calibrators.

Stability:

Reagent Integral:

Open use stability at 2-8°C was performed on one Reagent Integral Lot. At specified intervals, the stored opened kit was evaluated in parallel with a freshly opened kit. All testing was acceptable to 5 weeks. An open use stability of 4 weeks at 2-8°C is claimed. Open use stability on board the LIAISON® Analyzer was performed on one Reagent Integral Lot. At specified intervals, the opened kit was evaluated in parallel with a freshly opened kit. All testing was acceptable to 5 weeks. An open use stability of 4 weeks onboard the LIAISON® Analyzer is claimed.

Controls:

Open Use stability at 2-8°C was performed on one lot of LIAISON® Control Toxo IgM. At specified intervals, the stored opened controls were evaluated in parallel with a freshly opened vial of each control. All testing was acceptable to nine weeks. Open use stability at 2-8°C of eight weeks is claimed.

Expected Values:

Calibrator 1 is manufactured to have a concentration between 6.0-9.0 AU/mL.
Calibrator 2 is manufactured to have a concentration between 40-60 AU/mL.
The Negative Control is manufactured to a target value of 2.0 AU/mL.
The Positive Control is manufactured to have a target value 18.0 AU/mL.

d. Detection limit:

Not applicable - This assay is qualitative

e. Analytical specificity:

Cross-reactivity:

The cross-reactivity study for the LIAISON® Toxo IgM II assay was designed to evaluate potential interference from the presence of potentially cross-reactive antibodies or substances that may cause symptoms similar to or that may mimic toxoplasmosis infection. Only samples that were sero-positive for the cross reactant

and sero-negative for *Toxoplasma gondii* IgM by a commercially available Toxoplasma IgM assay were used to test for potentially cross-reactive organisms.

Cross-reactive organism	Number of samples tested	Comparator Toxo IgM Result	LIAISON [®] Toxo IgM		
			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

High Dose Hook Effect (false negative):

Analysis of high-dose hook effect was evaluated by testing three samples with *Toxoplasma gondii* IgM levels out-of-range > 160 AU/mL (i.e., 16 x minimum positive value). In this study, three samples with Toxo IgM levels were serially diluted in the assay Specimen Diluent to cover the entire assay range. The neat sample and dilutions were tested in triplicate using one kit lot on one instrument. The signal obtained was plotted versus the dilution. The samples resulted in calculated concentration values above the measuring range with no sample misclassification, indicating no hook effect was observed.

IgM Specificity:

Ten samples containing Toxoplasma IgM antibodies covering the assay range from value around cut-off level to clearly positive samples were treated with 10 mM dithiothreitol (DTT) to denature the IgM. These DTT treated samples were then tested in singlicate in parallel with the non-treated samples. All samples showed absence of IgM anti-Toxoplasma reactivity after treatment with DTT. These data demonstrate the specificity of the LIAISON[®] Toxo IgM II assay for IgM immunoglobulins.

Sample ID	LIAISON [®] Toxo IgM II assay Results (AU/mL)	
	Before DTT treatment	After DTT treatment
Sample-1	21.9	<3.0
Sample-2	15.6	<3.0

Sample-3	128	<3.0
Sample-4	60.7	<3.0
Sample-5	127	<3.0
Sample-6	26.9	<3.0
Sample-7	75.4	<3.0
Sample-8	25.3	<3.0
Sample-9	77.1	<3.0
Sample-10	36.7	<3.0

Interference:

Testing was performed to determine whether the presence of endogenous or exogenous substances may interfere with assay results. Three matched sample pools (2 negative and 1 positive) near the clinical decision point were tested neat and spiked with the respective interferent. Acceptance criteria were defined as the % change in signal must not be more than +10% and not change the qualitative result.

No interference was found 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

f. Assay cut-off:

The cutoff for the LIAISON® Toxo IgM II assay was set at an Arbitrary Unit value of 10.0 AU/mL based on European studies by testing 905 subjects from different populations (subjects sent to the lab for relevant testing, pregnant women, never infected subjects, subjects affected by autoimmune diseases, patients affected by various infectious diseases with similar symptoms, subjects with past infection, patients affected by acute infection and subjects with long-lasting IgM). The samples were tested in parallel with the LIAISON® Toxo IgM II assay and CE marked comparison methods. Consensus with the available clinical and serological data was applied to define the expected results.

The assay cut-off was validated in the United States during clinical studies by testing a prospective population of 804 samples from individuals sent to the laboratory for *Toxoplasma gondii* IgM testing, 201 pregnant women, and a retrospective population of 33 samples from individuals who had a positive *Toxoplasma gondii* IgM result. Based on the comparison studies this cutoff is appropriate for the LIAISON® Toxo IgM II assay. In the LIAISON® Toxo IgM II assay, a sample is defined as positive if

the Arbitrary Unit value is greater than or equal to 10.0 AU/mL, and defined as negative if the Arbitrary Unit value is less than 8.0 AU/mL. Samples with results greater than or equal to 8.0 AU/mL and less than 10.0 AU/mL are classified as equivocal.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Comparative Testing:

Prospective and Retrospective studies were performed to compare the performance of the LIAISON® Toxo IgM II assay to an FDA-cleared predicate device. The prospective study consisted of 804 samples (204 samples from US subjects, 600 samples from European subjects) from individuals who were sent to the laboratory for *Toxoplasma gondii* IgM testing and 201 pregnant women. The retrospective study consisted of 33 samples selected from individuals who had a positive *Toxoplasma gondii* IgM result by the predicate device.

Interpretation of Results:

Index	Results	Interpretation
< 8.0 AU/mL	Negative	Absence of detectable <i>Toxoplasma gondii</i> 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 <i>Toxoplasma gondii</i> specific IgM. If exposure to <i>Toxoplasma gondii</i> 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 <i>Toxoplasma gondii</i> IgM antibodies. A specimen with a positive result should be further tested for <i>Toxoplasma gondii</i> .

Prospective study:

The prospective US population, consisting of 204 individuals, was 96.1% Female (n=196) and 3.9% Male (n=8) ranging in age from 18 years to 42 years. There were 147 samples from patients where the age was unknown. The prospective European population consisted of 600 individuals. Age and gender for these samples are unknown. The prospective population of pregnant women consists of 201 females with ages ranging from 14 years to 44 years. There were 70 samples from subjects in the 1st trimester, 50 samples from subjects in the second trimester and 81 samples from subjects in the 3rd trimester of pregnancy. The agreement with 95% Confidence intervals for each prospective population is shown in the tables below:

Toxoplasma IgM Prospective US Population Comparison

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

Performance Summary: Toxoplasma IgM Prospective US Population Comparison

		Percent Agreement	Exact 95% Confidence Interval
Ne	203/203	100.0%	98.2 – 100.0%
Pos	0/1	NA	1.3 – 84.2%

Toxoplasma IgM Prospective European Population Comparison

LIAISON [®] Toxo IgM II	Comparative 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

Performance Summary: Toxoplasma IgM Prospective European Population Comparison

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

Toxoplasma IgM Pregnant Population

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

Performance Summary: Toxoplasma IgM Pregnant Population

		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 Diamedix Is-Toxoplasma IgM Capture result. There were 93.9% Females (n=31) and 6.1% Males (n=2) ranging in age from 15 to 47 years.

The agreement with 95% Confidence intervals for the pre-selected population is shown in the table below.

Retrospective study

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

Performance Summary: Retrospective study

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

CDC Panel study:

The CDC Toxoplasma 1998 Human Serum Panel is comprised of 100 frozen blind specimens (32 Toxoplasma IgM true positive samples and 65 Toxoplasma IgM true negative samples and three dilutions of three true Toxoplasma IgM positive samples). The panel was tested by LIAISON® Toxo IgM II assay at site #3. The results were submitted to the CDC (Reference Immunodiagnostic Lab, Biology and Diagnostic Branch Division of Parasitic Diseases) for data analysis. As communicated by the CDC, the LIAISON® Toxo IgM II assay correctly detected the 32 Toxoplasma IgM true positive samples (100 % agreement) and 65 Toxoplasma IgM true negative samples (100% agreement).

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The observed prevalence/ expected values using the LIAISON® Toxo IgM II assay was calculated for the prospective sample populations collected from US, European, and pregnant subjects. The samples from the US were from 8 males (3.9%) and 196 females (96.1%). There were 147 samples from patients where the age was unknown. Gender and ages for samples from the European subjects (n=600) are unknown. The pregnancy samples consisted of 201 females from the US with ages ranging 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 observed prevalence of the LIAISON® Toxo IgM II assay for each prospective population is as follows:

- US Population 0.0%
- European Population 16.7%
- Pregnant Women 1.5%

The prevalence results for each population are stratified by age and gender and are presented in the following tables:

Prevalence US population

Total	Age	Gender			LIAISON® Toxo IgM II Results			% Prevalence
		Male	Female	Unknown	Pos	Eqv	Neg	
0	<1	0	0	0	0	0	0	0
0	1-10	0	0	0	0	0	0	0
8	11-20	0	8	0	0	0	8	0

22	21-30	0	22	0	0	0	22	0
21	31-40	2	19	0	0	0	21	0
6	41-50	0	6	0	0	0	6	0
0	51-60	0	0	0	0	0	0	0
0	61-70	0	0	0	0	0	0	0
147	Unknown	6	141	0	0	0	147	0

Prevalence European Prospective Population

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

Prevalence Pregnant Women

		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

N. Instrument Name:

LIAISON[®] XL Analyzer

O. System Descriptions:

1. Modes of Operation:

The method for qualitative determination of specific IgM to *Toxoplasma gondii* is an antibody capture chemiluminescence immunoassay (CLIA). The principal components of the test are magnetic particles (solid phase) coated with IgG (mouse, monoclonal) to human IgM, *Toxoplasma gondii* antigen, and a conjugate of mouse monoclonal antibodies to *Toxoplasma gondii* 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 mouse monoclonal 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 the presence of *Toxoplasma gondii* IgM in calibrators, samples or controls.

All assay steps and incubations are performed by the LIAISON® XL Analyzer.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No _____

3. Specimen Identification:

Not Applicable

4. Specimen Sampling and Handling:

Not Applicable

5. Calibration:

DiaSorin's LIAISON® Toxo IgM II assay generates a continuous response (relative light units, RLU) which is used in sample grading to provide a qualitative (positive, negative, or equivocal) reportable result. The sample grading is based on the use of a calibration curve referenced to an IgM anti-Toxoplasma Internal Reference preparation and controlled by use of two calibrators (Calibrator 1 and Calibrator 2) provided in the Reagent Integral.

The calibrators are assayed by the user to transform the kit lot specific Master Curve into a Working Curve. The Master Curves are stored in the Radio Frequency Identification transponder (RFID Tag) of the Reagent Integral. Each Master Curve is generated by a mathematical elaboration of the data resulting from multiple testing (at least 10 runs) of an Internal Reference Preparation. During user calibration, the Calibrator results are used to create a Working Curve by mathematical adjustment of the Master Curve.

The Working Curve is then used to calculate sample results. (See Figure 6 for the calibration scheme.) The IgM anti-Toxoplasma Master Standards (e.g. points of the Internal Reference Preparation) were prepared from a pool of human serum or defibrinated plasma reactive for Toxoplasma IgM antibodies at established concentrations diluted in human serum or defibrinated plasma based matrix.

Calibrator doses are assigned with a specific Reagent Integral lot using the IgM anti-Toxoplasma Master Standard as the calibration curve in individual assays to assess the

AU/mL value. A reference panel of samples is tested and results obtained are compared between the IgM anti-Toxoplasma Master Standards and Calibrators and between expected results. If the calibrator AU/mL value is out of the expected range, the

Calibrators are adjusted by dilution or concentration. When the final AU/mL value is obtained, the Calibrators are tested for release. The lot-specific AU/mL values of Calibrators are stored in the Radio Frequency Identification transponder (RFID Tag). Calibrator 1 is manufactured to have a concentration between 6-9 AU/mL. Calibrator 2 is manufactured to have a concentration between 40-60 AU/mL. The calibrators are manufactured by diluting human serum/defibrinated plasma positive for IgM anti-Toxoplasma with a PBS/BSA buffer to obtain the target value. Calibrators and Controls are manufactured separately. Raw material segregation is maintained by the use of different part numbers for the stock solutions intended to manufacture the Calibrators and Controls.

The analyzer is calibrated in triplicate whenever one of the following conditions occurs: (see LIAISON® XL Analyzer Operator's manual):

- A new lot of Reagent Integral or a new lot of Starter Kit is used.
- The previous calibration was performed more than four weeks before.
- The analyzer has been serviced.
- Control values lie outside the expected ranges.

The process by which assay calibration was established and is maintained, is comprised of the following steps:

- Creation of the Internal Reference Preparation during the development phase. This step involves the selection of appropriate positive human serum/defibrinated plasma material, serial dilution with human negative serum/defibrinated plasma, and assignment of arbitrary AU/mL values appropriate for the range of LIAISON® Toxo IgM II kit. The final material is dispensed in aliquots and stored frozen. It is used as IgM anti-Toxoplasma Master Standards for calibration of reagents lots.
- Before exhaustion, the Internal Reference Preparation is restored by the selection of appropriate serum/defibrinated plasma material, serial dilution with human negative serum/defibrinated plasma, assignment of AU/mL values to the obtained points by calibration against the Internal Reference Preparation (ref. DiaSorin QC Procedure, CQst071).
- Generation of the Master Curve by multiple testing of the Internal Reference Preparation in at least ten Runs (ref. DiaSorin SOP 09.0245)
- Preparation of Kit Calibrators and assignment of calibrator values testing them against the Internal Reference Preparation. If necessary, Kit Calibrators concentrations are adjusted to achieve final target AU/mL (ref. DiaSorin QC

Procedure, (CQccl244).

- Adjustment of the lot-specific Master Curve on the basis of the Kit Calibrators results obtained by the user during instrument calibration. This Instrument Working Curve is used to calculate the patient sample results

6. Quality Control:

The LIAISON® Toxo IgM II assay procedure requires the running of controls in singlicate once per day of use. The LIAISON® Control Toxo IgM II consists of one negative and one positive serum based control. The controls were run at each site following the recommended procedure of once per day of use. If the controls failed, a new calibration was performed and controls were retested before beginning the testing for the day.

The following table shows a summary of the results:

Summary of Controls		Site 1		Site 2		Site 3	
ID	Range	Mean	%CV	Mean	%CV	Mean	%CV
Negative	0 - 4 AU/mL	NA	NA	NA	NA	NA	NA
Positive	10 -31 AU/mL	18.8	5.3%	17.2	13.2%	19.1	8.9%

The Negative control mean and % CV were not able to be calculated as the Negative control gave results of <3.0 AU/mL which is below the measuring range of the assay.

Based on the results, it was concluded that the LIAISON® Control Toxo IgM II provides an appropriate level of control for the LIAISON® Toxo IgM II assay.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.