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

A. 510(k) Number:

K132234

B. Purpose for Submission:

To obtain substantial equivalence for the DiaSorin LIAISON® IgG II and LIAISON® Control IgG II

C. Measurand:

Toxoplasma gondii IgG antibodies in human serum

D. Type of Test:

Chemiluminescence Immunoassay

E. Applicant:

DiaSorin Inc.

F. Proprietary and Established Names:

DiaSorin LIAISON® IgG II and LIAISON® Control IgG 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):

   DiaSorin LIAISON® Toxo IgG II assay
   The DiaSorin LIAISON® Toxo IgG II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® XL Analyzer® 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. This assay has not been cleared/approved by the FDA for blood/plasma donor screening. US Federal law restricts the 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.

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

2. Indication(s) for use:

   DiaSorin LIAISON® Toxo IgG II assay
   The DiaSorin LIAISON® Toxo IgG II assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® XL Analyzer® 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. This assay has not been cleared/approved by the FDA for blood/plasma donor screening. US Federal law restricts the 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.

   LIAISON® Control Toxo IgG II
   The LIAISON® Control Toxo IgG II (negative and positive) is intended for use as assayed quality control samples to monitor the performance of the LIAISON® Toxo IgG 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 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 LIAISON® XL 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, 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 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.

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 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 IgG II ([REF] 310706)

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Diamedix Is-Toxoplasma IgG ELISA

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

   K981498

3. Comparison with predicate:

Similarities		
Item	**Device (K132234)**	**Predicate (K981498)**
Intended Use	The LIAISON® Toxo IgG II assay is an immunoassay for the qualitative determination of specific IgG antibodies to *Toxoplasma gondii* in human serum specimens. 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. The assay has not been cleared/approved by the FDA for blood/plasma donor screening.	The Diamedix Is Toxoplasma IgG Test Kit is an immunoassay. For the qualitative, and quantitative, detection of IgG to Toxoplasma gondii in human serum. The results of this assay can be used as an aid in the assessment of the patient's immunological response to infection with T. gondii, and in the determination of immune status of individuals, including females of child-bearing years. This product has not been cleared/approved by the FDA for blood/plasma donor screening.
Measured Analyte	IgG antibodies to *Toxoplasma gondii*	IgG antibodies to *Toxoplasma gondii*
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

Differences		
Item	**Device (K132234)**	**Predicate (K981498)**
Assay Type	Chemiluminescent Immunoassay	Enzyme Assay
Calibration Standardization	E6 (National Health Laboratory, France 1987) standardized against WHO 2nd International Standard	WHO 3rd International Standard
Calculation of results	Qualitative Assay	Qualitative or Quantitative Assay
Unit of Measure	IU/mL	Index Value (Qualitative) IU/mL (Quantitative)
Cut-Off	≥ 8.8 IU/mL	1.10 Index Value (Qualitative) 50 IU/mL. (Quantitative)
Equivocal Zone	7.2 – 8.8 IU/mL	0.90 – 1.09 Index Value
Sample Size	20 µL	Minimum of 2 µL
Sample Handling/Processing	Automated	Manual or Automated
Assay Time	35 minutes	140 minutes
Controls	Provided separately	Included with kit
Conjugate	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative	Goat anti-human IgG labeled with horseradish peroxidase
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA microtiter plate reader)

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
- CSLI: M36-A: Clinical Use and Interpretation of Serological Tests for *Toxoplasma gondii*, Approved Guideline Vol. 24, No. 6

L. Test Principle:

This test is a Chemiluminescence Immunoassay (CLIA) – Immunoassay technology based on the emission of light as a result of a chemical reaction. 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 of the incubations, 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 RLUs and is indicative of the presence of anti-Toxo IgG in calibrators, samples or controls.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Precision was assessed by measuring repeatability at one site using two kit controls and six 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.

Precision

Sample ID	Sample N	Mean IU/mL	Within-Run		Within-Day		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Control*	80	<3.0	90.36*	5.6%*	89.13*	5.5%*	155.58*	9.6%*	200.8*	12.4%*
Pos 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.

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.

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
Neg Control*	480	<3.00	100.82*	6.4%*	87.89*	5.5%*	147.49	9.3%*	208.12*	13.1%*	320.20*	20.2%*
Pos 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.

b. Linearity/assay reportable range:

Not applicable – This assay is qualitative.

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

Traceability:

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).

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.

Unopened stored @ 2-8°C	Up to the stated expiration date
Opened stored @ 2-8°C	8 weeks
Opened stored on analyzer	8 weeks

Controls:

Unopened stored @ 2-8°C	Up to the stated expiration date
Opened stored @ 2-8°C	8 weeks

Samples:

Storage @ 2-8°C	7 days
Freeze/thaw	Up to 5 freeze/thaw cycles

Expected Values:

Calibrator 1 is manufactured to have a concentration between 10 - 20 IU/mL.
Calibrator 2 is manufactured to have a concentration between 200 - 400 IU/mL.
The Negative Control is manufactured to a target value less than 3.0 IU/mL.
The Positive Control is manufactured to have a target value of 22.0 IU/mL.

d. *Detection limit:*

Not applicable - This assay is qualitative.

e. Analytical specificity:

Cross-reactivity:

The cross-reactivity study for the LIAISON® Toxo IgG ll assay was designed to evaluate potential interference from the presence of potentially cross-reactive antibodies or substances and other viruses 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* IgG by a commercially available Toxoplasma IgG assay were used to test for potentially cross-reactive organisms.

Cross-reactive organism or condition	**Number of samples tested**	**Reference Toxo IgG Result**	**LIAISON® Toxo IgG II**		
			POS	**EQV**	**NEG**
Anti-HAV	5	Negative	0	0	5
Anti-HBc	5	Negative	0	0	5
Anti-VZV IgG	5	Negative	0	0	5
Anti-Rubella IgG	5	Negative	0	0	5
Anti-CMV IgG	10	Negative	0	0	10
Anti-EBV EBNA IgG	10	Negative	0	0	10
Anti-EBV VCA	10	Negative	0	0	10
Anti-HSV 1 IgG	10	Negative	0	0	10
Anti-HSV 2 IgG	5	Negative	0	0	5
Anti-ANA IgG	5	Negative	0	0	5
Anti-ds DNA IgG	2	Negative	0	0	2
Anti-Measles IgG	10	Negative	0	0	10
Anti-Mumps IgG	10	Negative	0	0	10
Treponema total antibodies	5	Negative	0	0	5
Anti-Parvo IgG	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

High Dose Hook Effect (false negative)*:*

Analysis of high-dose hook effect was evaluated by testing three samples with *Toxoplasma gondii* IgG levels out-of-range > 400 IU/mL. The samples resulted in calculated concentration values above the measuring range with no sample misclassification, indicating no hook effect was observed.

Interference:

Testing was performed to determine whether the presence of endogenous or exogenous substances may interfere with assay results. Three matched sample pools containing antibodies to Toxoplasma IgG 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 IgG ll 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	20 mg/dL
Albumin	6000 mg/dL
Cholesterol	510 mg/dL

f. Assay cut-off:

The cutoff for the LIAISON® Toxo IgG ll assay was set at an International Unit value of 8.8 IU/mL based on European studies by testing 1000 subjects from different populations. The samples were tested in parallel with the LIAISON® Toxo IgG II assay and CE marked comparison methods. Consensus between the methods as well as 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* testing and 202 pregnant women. Ten samples resulted in an equivocal interpretation on the comparator test and therefore, were not used in analysis. Based on the comparison studies this cutoff is appropriate for the LIAISON® Toxo IgG II assay. In the LIAISON® Toxo IgG II assay, a sample is defined as positive if the International Unit value is greater than or equal to 8.8 IU/mL, and defined as negative if the International Unit value is less than 7.2 IU/mL. Samples with results greater than or equal to 7.2 IU/mL and less than 8.8 IU/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 IgG 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* testing and 202 pregnant women.

The retrospective study consisted of 42 samples selected from individuals who had a positive *Toxoplasma gondii* IgG result by the predicate device.

Interpretation of Results:

Index	Results	Interpretation
< 7.2 IU/mL	Negative	Absence of detectable *Toxoplasma gondii* IgG antibodies. A negative result does not always rule out acute infection. The test usually scores negative in infected people 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 IU/mL - < 8.8 IU/mL	Equivocal	The equivocal sample should be repeat tested. If 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 my be postulated.

Prospective study
The prospective US population consisting of 204 individuals were 96.1% female (n=196) age and 3.9% male (n=8) ranging in age from 18 to 42 years. There were 147 samples from subjects 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 202 females with ages ranging from 14 years to 44 years. There were 71 samples from subjects in the 1st trimester, 50 samples form 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 IgG Prospective US Population Comparison

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%

Toxoplasma IgG Prospective European Population Comparison

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%

Toxoplasma IgG Pregnant Population Comparison

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 the Diamedix Is-Toxoplasma IgG ELISA. There were 95.2% females (n=40) and 4.8% males (n=2) ranging in age from 0 years to 47 years.

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

Toxoplasma Retrospective Population Comparison

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%	91.6 – 100.0%

CDC Study Panel:

The CDC Toxoplasma 1998 Human Serum Panel panel is comprised of 100 frozen blind specimens (70 Toxoplasma IgG true positive samples, and 30 Toxoplasma IgG true negative samples). The panel was tested by LIAISON® Toxo IgG ll assay. The results were submitted

to the CDC (Reference Immunodiagnostic Lab, Biology and Diagnostic Branch Division of Parasitic Diseases) for data analysis. As communicated by CDC, the LIAISON® Toxo IgG II assay correctly detected the 70 Toxoplasma IgG true positive samples (100% Sensitivity) and the 30 Toxoplasma IgG true negative samples (100% Specificity).

4. Clinical cut-off**:**

Not applicable

5. Expected values:

The observed prevalence of the LIAISON® Toxo IgG II assay was calculated from the prospective sample populations collected from US, European, and pregnant subjects. The samples from US subjects (n=204) consisted of 8 males (3.9%) and 196 females (96.1%). Known ages ranged from 18 years to 42 years. There were 147 samples where age was unknown. Gender and ages for samples from the European subjects (n=600) are unknown. The pregnancy samples consisted of 202 females with ages ranging 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 observed prevalence of the LIAISON® Toxo IgG II assay for each prospective population is as follows:

- US Population 10.3%
- European Population 57.0%
- Pregnant Women 5.9%

N. Instrument Name:

LIAISON® XL Analyzer

O. System Descriptions:

1. Modes of Operation:

The method for qualitative determination of IgG antibodies to Toxoplasma gondii (anti-Toxo IgG) is an indirect chemiluminescence immunoassay (CLIA). 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.

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 ___√_____ or No _____

3. Specimen Identification:
   Not Applicable

4. Specimen Sampling and Handling:
   Not Applicable

5. Calibration:

   DiaSorin's LIAISON® Toxo IgG 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 a calibration curve referenced to National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987), standardized against WHO 2nd International Standard (1980) Reference preparation. Typical dose-response curve of any assay kit lot is described by a dedicated master curve.

   The two calibrators in the kit are assayed by the user to transform the kit lot specific Master Curve into a Working Curve, describing the testing conditions specific for the given testing session. From the working curve, doses for the unknown samples are calculated. For each lot, the Master Curve is stored in the Radio Frequency Identification transponder (RFID Tag) of the Reagent Integral. Master curve is defined for each lot during the quality control of the batch. In the same process, calibrators are checked for compliance with expected concentration and adjusted if not compliant. In the same testing session actual titer is assessed. A final trimming of the titer is then achieved targeting established reference doses of the Quality Control Panel. Calibrator 1 is manufactured targeting a concentration between 10-20 IU/mL. Calibrator 2 is manufactured targeting a concentration between 200-400 IU/mL. Calibrators are manufactured from serum or defibrinated plasma units at a proper concentration of IgG anti-Toxoplasma, and then diluted in buffer to reach desired titer.

In five different experiments, a calibration panel, batch calibrators and a quality control panel are assayed in order to define the master curve and titration of the calibrators:

1. Calibration panel determines the working curve of each of the five experiments and allows the calculation of the doses associated to the batch calibrators;
2. Doses of the batch calibrators are assessed in respect to the target range. Calibrators are corrected by dilution or concentration as needed;
3. From the five working curve a master curve for the specific batch is calculated;
4. Doses of the calibrators are used to calculate results for the quality control panel from the defined master curve: a fine trimming of the calibrator's dose is possible in order to optimize response on the quality control panel in respect to reference established ranges.

The calibration panel is made of two major components:

1. A Full Standardization Curve: Full Standardization Curve is obtained as an Internal Standard Curve from a pool of human serum and/or defibrinated plasma at high titer, serially diluted in a negative and/or defibrinated plasma pool. It is calibrated versus an Internal Primary Standard Curve, in turn referenced to the National Standard Serum for Toxoplasmosis E6 (National Health Laboratory, France, 1987) Primary Curve. This is obtained by direct reconstitution and dilution of the E6 Standard;
2. An accuracy panel: a set of samples at various doses along the assay range. Reference established doses of members of the accuracy panel are defined in a specific qualification session.

The Calibration panel is used to determine the typical dose-response curve of the assay accounting for the behavior of both the reference standard and true specimens.

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 fall outside the expected ranges.

Calibrators and controls are manufactured separately. Raw material segregation is also maintained by the use of different part numbers for the stock solutions intended to manufacture the calibrators and controls.

6. 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 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 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 be within the expected ranges: whenever one of the controls results fall outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration fall repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If the control values fall outside the expected ranges, patient results must not be reported.

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.