510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k061247

B. Purpose for Submission:
Addition of the DiaSorin LIAISON® Treponema Assay to the LIAISON® Chemiluminescence Assay

C. Measurand:
To detect antibodies to *T. pallidum*

D. Type of Test:
Chemiluminescence immunoassay

E. Applicant:
DiaSorin Inc.

F. Proprietary and Established Names:
DiaSorin LIAISON® Treponema Assay

G. Regulatory Information:

1. Regulation section:
   CFR 866.3830 Enzyme-linked immunoabsorption assay, *Treponema pallidum*

2. Classification:
   II

3. Product code:
   LIP

4. Panel:
   83 Microbiology

H. Intended Use:

1. Intended use(s):
   The LIAISON® Treponema assay uses chemiluminiscence immunoassay (CLIA) technology for the qualitative detection of total antibodies of any class (IgG/IgM) directed against *Treponema pallidum* in human serum. The presence of antibodies to *Treponema pallidum* specific antigen, in conjunction with non treponemanal laboratory tests and clinical findings, may aid in the diagnosis of syphilis infection. The LIAISON® Treponema assay is not intended for use in

screening blood or plasma donors.

2. Indication(s) for use:
   In conjunction with nontreponemal laboratory tests and clinical findings, may aid in the diagnosis of syphilis infection.

3. Special conditions for use statement(s):
   For prescription use only

   The LIAISON® Treponema assay is not intended for use in screening blood or plasma donors.

   Warning: A positive result is not useful for establishing a diagnosis of syphilis. In most situations, such a result may reflect prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease.

   Warning: "When a sample result displays the exclamation mark (!) flag, the result obtained lies below the assay's signal range**.** The sample should be retested and graded negative if the result is still below the signal range upon retest."

   "Assay interference due to circulating antibodies against Hepatitis A viruses, yaws, pinta and leptospirosis have not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents."

4. Special instrument requirements:
   LIAISON® Chemiluminescence Analyzer

I. Device Description:

The LIAISON® Treponema assay is an *in vitro* diagnostic device consisting of reagents provided in individual compartments within a plastic container called the **Reagent Integral**. The assay configuration for the LIAISON® Treponema assay allows for the performance of 200 tests.

Reagent Integral Composition:

a. Magnetic Particles
b. 2 Calibrators that are human serum or defibrinated plasma
c. Specimen diluent
d. Conjugate which is a Tp17 DNA recombinant protein (obtained in *E. coli*)

J. Substantial Equivalence Information:

1. Predicate device name(s):
   CAPTIA™ Syphilis (T. pallidum)-G

2. Predicate 510(k) number(s):
k014233

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
1. Intended Use	Qualitative detection of antibodies to *T. pallidum* in human serum	Qualitative detection of antibodies in serum specimens
2. Controls	2 (Negative and Positive)	2 (Negative and High Titer Positive control)
3. CutOff	1.0 Index	Same
4. Equivocal zone	0.9 – 1.1	Same
5. Reagent Storage	2 - 8°C, On board or in Refrigerator	2 - 8°C Refrigerator only
6. Laboratory Use	Clinical diagnostic Confirmation Clinical Laboratory Screen	Same

Differences		
Item	Device	Predicate
1. Antibody identification	Qualitative detection of total antibodies of any class (IgG/IgM) directed against *T. pallidum* in humans serum	Qualitative detection of IgG antibodies to *T. pallidum* in serum specimens
2. Calibrators	2	Low titer reactive control in duplicate (mean value is assay cutoff)
3. Sample Matrix	Serum	Serum and Plasma
4. Type of Assay	Indirect Chemiluminescence Immunoassay	Enzyme Immunoassay (EIA)
5. Sample Handling/Processing	Automated	Manual or in conjunction with a qualified Automatic or Semi-automatic processor/liquid handling system
6. Antigen Used	DNA-Tp17 Recombinant antigen (obtained in *E. coli*)	Whole cell sonicated *T. pallidum* antigens, Nichols strain

Differences		
Item	Device	Predicate
7. Detector	Tp17 DNA recombinant protein (obtained in *E. coli*) conjugated to an isoluminol derivative	Horseradish-peroxidase (HRP) labeled mAb
8. Capture Reagent	Magnetic particles coated with Tp17 DNA recombinant protein (obtained in *E. coli*)	Microtitration wells coated with whole-cell sonicated *T. pallidum* antigens, Nichols strain
8. Calibration	Two-point verification (in triplicate) of stored 10 point master curve	Low titer reactive control in duplicate (mean value is assay cutoff)
9. Measurement system	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA Microtiter plate reader)
10. Total incubation	40 minutes	2.5 hours (150 minutes)

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

Not Applicable

L. Test Principle:

Recombinant antigens specific for *T. pallidum* are used for coating the magnetic particles (solid phase) and are used in the tracer when linked to an isoluminol derivative (isoluminol-antigen conjugate).

All assay steps and incubations are performed by the LIAISON® Analyzer, with the exception of initial magnetic particle resuspension.

During the incubation step antibodies present in the calibrators, samples or controls bind to the solid phase. The conjugate reacts with the antibodies already bound to the solid phase. After the incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash Chemiluminescence reaction is induced. The light signal and hence the amount of isoluminol-antigen conjugate is measured by a photomultiplier as relative light units (RLU) and is indicative of total antibodies to *Treponema pallidum* present in calibrators, controls or samples.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

Reproducibility testing was performed at 3 US sites, using four replicates per run in one run per day during five operating days and a different lot of the LIAISON® Treponema Reagent Integral kit lot was used for each site. A coded panel comprised of 9 frozen "engineered" serum samples and the LIAISON® Treponema Serum controls were included in the study. The panel consisted of 4 samples near the cut-off, 2 negative, 2 low positive and 1 positive. The table below reflects the summary of results of the reproducibility study among the 3 sites.

Reproducibility Index

Sample ID	N	Mean Index	Within run %CV	Between run %CV	Between site %CV	Overall %CV Index	Overall SD Index
1001	60	0.94	4.90	4.19	5.76	7.91	0.07
1002	60	1.07	2.86	3.99	4.54	6.07	0.06
1003	60	1.42	3.03	3.57	7.02	8.08	0.11
1004	60	0.95	3.86	3.60	6.76	7.43	0.07
1005	60	0.99	2.62	6.22	6.81	8.60	0.09
1006	58	1.26	1.89	3.93	6.70	6.93	0.09
1007*	58	1051	7.78	5.91	13.65	14.87	157
1008*	60	1047	11.17	8.95	4.32	23.66	248
1009	60	13.13	3.11	3.75	4.87	6.93	0.91
NC	60	0.25	7.02	12.16	17.76	20.06	0.05
PC	60	5.06	2.70	4.94	5.76	7.28	0.37

*Sample numbers 1007 and 1008 were negative samples that read below the assay range and Index values were nondetectable. Relative Light Units (Signal) were used in the precision summary table.

b. Linearity / Assay Reportable Range

The assay range is 0.1 to 70 Index. Negative results can be below the assay range, these samples are reported as <0.1 Index and positive samples above 70 are reported as >70 Index.

Samples containing extremely high antibody concentrations (prozone) when tested in a one-step sandwich assay may exhibit a hook effect and mimic concentrations that are lower than expected. Analysis of the hook effect for the LIAISON® Treponema assay was evaluated by testing 3 high titered samples positive for total antibodies against Treponema pallidum. All 3 neat samples had Index values >70 (the high end of the reportable range). The samples were serially diluted and the results of the dilutions gave expected results with the sample Index decreasing in value with each dilution. An approximation of the total Index value by correcting for the dilution factor (i.e.1:2) shows no high dose hook effect in samples with an anti-Treponema Index between 73.4 and 132. Two other samples that had high antibody concentrations, but did not read above the assay range of 70, were also analyzed. Both of these samples were serially diluted and results obtained gave expected results with the sample Index decreasing in value

with each dilution as well.

A statement concerning High-Dose hook effect will be included in the Instructions for Use "Whenever samples containing extremely high antibody concentrations are tested in a one-step sandwich method, the hook effect can mimic concentrations lower than expected. Analysis of hook effect was done by serially diluting three high-titered samples positive for total antibodies to *Treponema pallidum.* All neat samples had an Index value above the measuring range (>70), which would be expected with high-titer sera. An approximation of the Index value by correcting for the dilution factor (i.e. 1:2) shows no high dose hook effect was observed in samples with an anti-Treponema Index of approximately 132."

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

The LIAISON® Treponema Serum controls consist of 1 negative and 1 positive and are intended for use as assayed quality control samples to monitor the performance of the LIAISON® Treponema assay. Control performance has not been established for matrices other than serum. The reference range of each control is reported on the corresponding vial label.

LIAISON® Treponema Serum controls

NEGATIVE CONTROL 2 vials – 2mL each	Human Serum or defibrinated plasma negative for antibodies to *Treponema pallidum* Preservatives: 0.1% ProClin® 300
POSITIVE CONTROL 2 vials – 2mL each	Human Serum or defibrinated plasma **positive/reactive for IgG/IgM antibodies** to *Treponema pallidum* in diluted in human serum negative for antibodies to *Treponema pallidum.* Preservatives: 0.1% ProClin® 300

QC was performed once each day of use with results in the expected range most of the time.

Summary of Controls

	Acceptance	Site 1		Site 2		Site 3	
	Range (Index)	Mean	Total %CV	Mean	Total %CV	Mean	Total %CV
Serum Controls							
Negative	<0.8	0.23	11.24	0.22	23.72	0.3	5.75
Positive	3 – 8	5.24	4.46	4.73	7.81	5.23	2.07

European Buffer based control results were provided. However, this data was not evaluated because we do not have sufficient information to assess this matrix for control material.

On Board stability was evaluated using one lot that was left on board the instrument, in the refrigerated area (12 - 19°C), for 28 days. The kit performance was evaluated without any integral calibration, testing positive and negative

samples. The data obtained showed results within the expected range. A claim of four weeks on-board stability has been included in the package insert.

Calibration stability study was also performed using one lot. After initial calibration of newly opened reagent integral, samples were tested and integral was stored on board the instrument, in the refrigerated area. At established intervals, positive and negative samples were tested using the stored integral and original calibration. 2-week calibration stability is claimed in the insert.

Open vial stability was evaluated using 2 lots of LIAISON® Treponema Serum controls. These controls were placed on the analyzer and tested in replicates of 6 at specified time intervals. 4-week open vial stability will be used.

d. Detection limit:

This is a qualitative assay (Pos, Neg, or Equivocal Results) so there is no limit of detection. Negative samples can be read below the low end of the assay range of 0.1 and are given a <0.1 result. There is a low limit of 350 RLU, for the LIAISON® Treponema Assay. Any sample below this limit will have an error code (!) next to the result and sample must be repeated.

The following **warning statement** has been added to the package insert under the Interpretation of results section to address results below the low limit of the assay range "When a sample result displays the exclamation mark (!) flag, the result obtained lies below the assay's signal range**.** The sample should be retested and graded negative if the result is still below the signal range upon retest."

e. Analytical specificity:

Cross-Reactivity Study

Two hundred forty four samples were tested for cross-reactivity; which was performed at DiaSorin S.p.A., DiaSorin, Inc. and at European clinical laboratories. Samples with negative or equivocal results by the predicate device and the LIAISON® Treponema Assay were considered not to demonstrate cross reactivity. Cross reactivity could not be determined in samples that returned positive values by the reference method as no additional testing of the samples by darkfield or DFA-TP or equivalent methods was done. The table below reflects the cross reactivity study.

Summary of Cross reactivity testing

Organism / condition	**# of Samples**	**Positive Result**
Borrelia f. IgG (European strain)	3	0/3
Borrelia f. IgM (European strain)	10	0/10
Borrelia f. IgG /IgM (European strain)	2	0/2
CMV IgG	4	0/4
CMV IgM	2	0/2
Rubella IgG	4	0/4

Rubella IgM	2	0/2
Toxoplasma gondii IgG	4	0/4
Toxoplasma gondii IgM	3	0/3
VZV IgG	5	0/5
EBV VCA IgM	12	0/12
Streptococcus β haemolyticus	10	0/10
ANA	16	0/16
RF	10	0/10
Borrelia burgdorferi (US strain)	20	1/20
Systemic Lupus Erythematosis (SLE)	23	0/23
HIV	95	0/95
E. coli	3	0/3
HBsAg	3	0/3
Anti-HBs	5	0/5
HCV	5	0/5
Total	244	1/244

Samples that are positive for antibodies against Hepatitis A virus and negative for *T. pallidum* and samples for yaws, pinta and leptospirosis are unavailable therefore the following **limitation statement** will be included "Assay interference due to circulating antibodies against Hepatitis A viruses, yaws, pinta and leptospirosis have not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents."

Interference Study

Testing was performed to determine whether the presence of hemoglobin, bilirubin or triglycerides will interfere with assay results. Ten negative and 10 positive samples at different level of antibodies to *T. pallidum* were spiked with a known amount of an interfering substance with no significant difference between the two sample types.

Interfering Substances Data

Substance	Expected Range	Tested Concentration
Hemoglobin	<3 mg/dL	1000 mg/dL
Bilirubin	0.2 to 1.0 mg/dL	20 mg/dL
Triglycerides	40 to 160 mg/dL	3000 mg/dL

Influence of Sample Storage

1) Freeze-thawing

The effect of freeze-thawing sample was evaluated using 4 negative and 4 positive samples. Although samples cycled through freeze-thaws did not have any apparent effect on the assay performance, the following statement has been included in the package insert to follow CLSI's recommendation "Samples should not be repeatedly frozen and thawed. Self-defrosting freezers are not recommended for sample storage."

2) Sample Storage at 2 - 8° C

In this study, 4 negative and 4 positive samples were used to demonstrate the effect of sample storage at 2 - 8° C with no effect on assay performance. The following statement has been included in the submission "Samples may be kept at 2 - 8°C for no longer than 7 days, otherwise they should be dispensed in aliquots and stored deep-frozen (-20°C or below)."

Carry Over Effect

Sample carry-over has been evaluated by testing a negative sample before and after a high positive sample with no observable effect.

f. Assay cut-off:

The cut-off for the LIAISON® Treponema Assay was determined during European clinical trials using 3,625 samples. An analysis of the cumulative frequency distributions (ROC analysis) was performed on the LIAISON® Treponema Assay results for the 1000 non selected routine laboratory samples, 131 characterized syphilis samples and 2494 donor samples. The relevant cumulative frequency distribution graph was generated by calculating sensitivity from the 131 (positive) characterized syphilis samples and the 48 (positive) routine laboratory samples while specificity was determined from 952 (negative) routine laboratory samples, and the 2494 blood donors. Analysis of the data showed that the cut-off value discriminating between the presence and the absence of *T. pallidum* total antibodies has an Index value of 1.0 with an equivocal zone between 0.9 and <1.1.

The cut-off was verified in the United States during clinical trials where 97% gave Index values less than 0.5 and 97.9% gave Index values greater than 1.4.

2. Comparison studies:

a. Method comparison with predicate device:

Clinical testing was performed at 3 US sites - 2 external and 1 in-house testing using frozen prospective and retrospective samples received from sample procurement organizations that have been supplied with the selection criteria.

The clinical study was divided into 2 groups where in Study #1 LIAISON® Treponema Assay was utilized as a clinical laboratory screen (diagnostic test), and Study #2 LIAISON® Treponema Assay was utilized as a diagnostic confirmatory test.

All samples were tested with the LIAISON® Treponema Assay and the predicate device, Trinity CAPTIA™ Syphilis (*T. pallidum*) – G. Discordant samples were further tested as described in the Use of Treponemal Tests to Screen for Syphilis.

Study #1 LIAISON® Treponema Assay as a clinical laboratory screen (diagnostic test). Treponemal test followed by a non treponemal test.

A. Medically Diagnosed Syphilis Samples

Fifty one US samples of medically diagnosed syphilis were tested with the LIAISON® Treponema assay and the Trinity CAPTIA™ Syphilis (*T. pallidum)* –G with the following results.

US Data for Medically Diagnosed Syphilis

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	47		1*	48
Negative	1*	1	1*	3
Equivocal				
Total	48	1	2	51

Three discordant samples resolved upon further testing.

	Percent Agreement		Exact 95% Confidence Interval
Positive	97.9 %	(47/48)	89.0 – 99.9%
Negative	100%	(1/1)	2.5 – 100%
Overall	94.1%	(48/51)	83.8 – 98.8%

One hundred twenty seven samples were tested in Europe with the following results.

European data for Medically Diagnosed Syphilis

LIAISON® Treponema Assay	Syphilis – G ELISA Assay			Total
	Positive	Negative	Equivocal	
Positive	118	5*	3*	126
Negative				
Equivocal	1*			1
Total	119	5	3	127

European	Percent Agreement		Exact 95% Confidence Interval
Positive	99.2 %	(118/119)	95.0 - 99.9%
Negative	0%	(0/5)	0.0 – 45.0%
Overall	92.9%	(118/127)	88.0 – 96.2%

B. Samples sent to the Laboratory for Syphilis Testing

Nine hundred ninety-nine samples were collected in the State of New York and

the Southeastern United States from patients sent to the laboratory for syphilis testing. Testing of these samples was split between 2 US sites. The table below shows comparison of the new and the predicate devices.

Samples sent for Laboratory testing

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	22	9*	10*	41
Negative	18*	909	30*	957
Equivocal		1*		1
Total	40	919	40	999

Even after additional testing following the Algorithm to Screen for Syphilis: Treponema Test as Screen, there were 12 samples that remained unresolved.

	Percent Agreement		Exact 95% Confidence Interval
Positive	55%	(22/40)	38.6 – 70.7%
Negative	98.9%	(909/919)	98.0 – 99.5%
Overall	93.2%	(931/999)	91.4 – 94.7%

C. HIV Positive Samples

Two hundred HIV positive samples were collected from patients in the southeastern US with the following results. Testing of these samples was split between 2 US sites.

HIV Positive Samples

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	69	4*	2*	75
Negative	20*	100	3*	123
Equivocal	2*			
Total	91	104	5	200

There were 11 samples that were not resolved even after additional testing with RPR and TP-PA.

	Percent Agreement		Exact 95% Confidence Interval
Positive	75.8%	(69/91)	65.8 – 83.5%
Negative	96.2%	(100/104)	90.4 – 98.9%
Overall	84.5%	(169/200)	78.7 – 89.2%

D. Pregnancy Samples

Two hundred pregnancy samples (first, second and third trimester) were collected in the Mid-Atlantic and Northeastern US with the following results. Testing was done in house.

Pregnancy Samples

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	4			4
Negative		192	4*	196
Equivocal				
Total	4	192	4	200

All 4 discordant samples resolved on further testing.

	Percent Agreement		Exact 95% Confidence Interval
Positive	100%	(4/4)	39.8 - 100%
Negative	100%	(192/192)	98.1 – 100%
Overall	98.0%	(196/200)	95.0 – 99.5%

E. Apparently Healthy Adults

Nine hundred ninety-two unselected samples were collected from apparently healthy adults in the Southeastern US. The table below reflects the results between the LIAISON® Treponema Assay and the Trinity CAPTIA™ Syphilis (*T. pallidum*) – G. Testing of samples was split between 2 US sites.

Apparently Healthy Adults

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	54	6*	3*	63
Negative	32*	881	16*	929
Equivocal				
Total	86	887	19	992

Seventeen samples remained unresolved even after additional testing.

	Percent Agreement		Exact 95% Confidence Interval
Positive	62.7%	(54/86)	51.7 – 73.0%
Negative	99.3%	(881/887)	98.5 – 99.8%
Overall	94.2%	(935/992)	92.6 – 95.6%

Study #2 LIAISON® Treponema Assay as a diagnostic confirmatory test.

A. RPR/VDRL Positive Samples

Two hundred four samples collected from the Northeastern and Southeastern regions of the United States were tested between the new and the predicate devices with the following results.

RPR/VDRL Positive Samples

LIAISON® Treponema Assay	**Syphilis G ELISA Assay**			Total
	Positive	Negative	Equivocal	
Positive	200		1*	201
Negative		2		2
Equivocal	1*			1
Total	201	2	1	204

There were 2 samples from the RPR positive sample with a discordant result however 1 sample was QNS for testing and the other resolved on further testing.

	Percent Agreement		Exact 95% Confidence Interval
Positive	99.5%	(200/201)	98.2 – 100%
Negative	100%	(2/2)	15.8 – 100%
Overall	99.0%	(202/204)	97.3 - 100%

b. Matrix comparison:
Not applicable

3. Clinical studies:

a. Clinical Sensitivity:
Not applicable

b. Clinical specificity:
Not applicable

4. Clinical cut-off:
Not applicable

5. Expected values/Reference range:

Negative Index Value <0.9

Positive Index Value ≥ 1.1

Equivocal Index Value 0.9 – 1.1

Equivocal samples must be retested in order to confirm the initial result. Samples which are positive at the second test should be considered positive. Samples which are negative at the second test should be considered negative. A second sample should be collected and tested no less than one week later when the result is repeated equivocal.

Samples giving equivocal or positive results must be supplemented with a quantitative nontreponemal test (RPR or VDRL).

N. Proposed Labeling:

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

O. Conclusion:

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