

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

A. 510(k) Number:

K091361

B. Purpose for Submission:

Substantial equivalence determination for the Immulite 2000 Syphilis Screen test system

C. Measurand:

To detect antibodies to *Treponema pallidum* (*T. pallidum*)

D. Type of Test:

A solid phase, one-step chemiluminescent immunoassay

E. Applicant:

Siemens Healthcare Diagnostics, Inc.

F. Proprietary and Established Names:

Immulite® 2000 Syphilis Screen test system

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.3830, *Treponema pallidum* treponemal test reagents

2. Classification:

Class II

3. Product code:

LIP - Enzyme linked immunoabsorption assay, *Treponema pallidum*

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use:

The Immulite 2000 syphilis screen test is a treponemal testing procedure for the qualitative detection of antibodies to *Treponema pallidum* in human serum or heparinized plasma on the Immulite 2000 analyzer as an aid in the diagnosis of syphilis. The Immulite 2000 syphilis screen is not intended for use in screening blood or plasma donors.

2. Indications for use:

The Immulite 2000 syphilis screen test is a treponemal testing procedure for the qualitative detection of antibodies to *Treponema pallidum* in human serum or heparinized plasma on the Immulite 2000 analyzer as an aid in the diagnosis of syphilis. The Immulite 2000 syphilis screen is not intended for use in screening blood or plasma donors.

3. Special condition for use statement:

For prescription use only.

4. Special instrument requirements:

Immulite 2000 Random Access Analyzer.

I. Device Description:

The device is a kit consisting of a bead pack of 200 beads coated with purified recombinant *T. pallidum* 17 antigen (Tp17); a syphilis screen reagent wedge consisting of 11.5 ml. of alkaline phosphatase conjugated to purified recombinant Tp 17 antigen; a syphilis screen adjustor consisting of one vial of lyophilized human serum with antibodies reactive to *T. pallidum* and syphilis screen controls – positive and negative . Kit components supplied separately include a chemiluminescent substrate, a probe wash, a probe cleaning kit and disposable reaction tubes.

J. Substantial Equivalence Information:

1. Predicate device name:

- DiaSorin Liaison® Treponema Assay

2. Predicate 510(k) number:

- K061247

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Immulite 2000 Syphilis screen test is a treponemal testing procedure for the qualitative detection of antibodies to <i>Treponema pallidum</i> in human serum or heparinized plasma	The LIAISON Treponema assay uses chemiluminescent immunoassay technology for the qualitative detection of total antibodies directed against <i>Treponema pallidum</i> in human serum
Assay type	Enzyme labeled, one step chemiluminescent immunoassay	One step sandwich chemiluminescent immunoassay
Cut-Offs	≥ 1.1 Reactive 0.9 to <1.1 Indeterminate < 0.9 non-reactive	≥ 1.1 Positive 0.9 to < 1.1 equivocal < 0.9 negative

Differences		
Item	Device	Predicate
Sample volume	100µl	220 µl
Capture detection Antigen/Antibody	Beads coated with purified recombinant <i>Treponema pallidum</i> p17 (TpN17) antigens are linked to enzyme conjugated purified recombinant <i>T. pallidum</i> p17 antigen in the reagent	Magnetic particles coated with Tp17 DNA recombinant protein specific for <i>T. pallidum</i> are linked to an isoluminol antigen conjugate

K. Standard/Guidance Document Referenced:

CLSI – EP 07-A2 : Interference testing in Clinical Chemistry
CLSI – EP 05: Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

Immulite 2000 Syphilis Screen is a solid-phase, one-step chemiluminescent enzyme immunoassay. The solid phase (beads) is coated with purified recombinant *Treponema pallidum* p17 (Tp17) antigen. The liquid phase consists of alkaline phosphatase (bovine calf intestine) conjugated to purified recombinant *Treponema pallidum* p17 (Tp17) antigen.

Patient sample and the reagent are incubated together with the coated beads for 30 minutes. During this time, total antibody to *Treponema pallidum* in the sample forms the antigen sandwich complex with purified recombinant *Treponema pallidum* p17 (Tp17) antigen on the bead and enzyme conjugated purified recombinant *Treponema pallidum* p17 (Tp17) antigen in the reagent. Unbound patient sample and enzyme conjugate are then removed by centrifugal washes. Finally, chemiluminescent substrate is added to the reaction tube containing the bead and the signal is generated in proportion to the bound enzyme.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility testing was performed using two different reagent lots at all testing sites for five days, twice each day. There were 4 replicates of each sample, quality control materials that are routinely provided with the kit (positive / negative controls) and 5 endogenous samples (low negative, high negative, low positive, high positive, and a sample around the cutoff) from pooled serum. Additionally, the assay adjustor was run as an unknown. This resulted in a yield of 40 replicates of each sample per lot at each site. Reproducibility of the Immulite syphilis screen as measured by between-site CV ranged from 7.1 % to 22.6% for Lot 1 and from 6.1% to 29.7% for Lot 2.

b. Linearity/assay reportable range:

Not applicable.

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

Not applicable.

d. Detection limit:

The Master Cutoff of the assay was determined from representative samples to achieve optimal sensitivity and specificity for the assay. The cutoff is set equal to the average counts per second of the Adjustor multiplied by the Curve Parameter 1. The result for a sample is reported as “Reactive” if the sample’s counts are above the indeterminate range, and “Nonreactive” if below this range. The result is reported as “Indeterminate” if the counts per sec. for that sample fall within $\pm 10\%$ of the cut off.

e. Analytical specificity:

Interference Study: Interference testing was conducted using serum and plasma per CLSI EP7A, Interference Testing in Clinical Chemistry. The following results were obtained:

- Hemoglobin: No effect was observed up to 592.6 mg/dL of hemoglobin;
- Bilirubin: no effect was observed up to 20 mg/dL of conjugated or unconjugated bilirubin;
- Triglycerides: No effect was observed up to 3000 mg/dL of triglycerides.

Cross-Reactivity Study: To test for possible cross-reactivity, multiple patient samples containing different concentrations of potentially cross-reactive antibodies were analyzed with the Immulite 2000 Syphilis Screen test.

Organism	Total # Samples	# Reactive/ Total # Samples
Cytomegalovirus IgG	10	1/10
Epstein-Barr Virus	10	1/10
Anti-Nuclear Antibodies	10	0/10
Herpes Simplex Virus IgG	10	1/10
Rubella IgG	10	1/10
Toxoplasma IgG	10	0/10
Rheumatoid Factor (anti-Fc)	10	0/10
Borrelia burgdorferi IgG (US strain)	10	2/10
Borrelia burgdorferi IgG (European strain)	10	0/10
Borrelia burgdorferi IgM (European strain)	10	0/10
Anti-Hepatitis B Surface Antigen	10	1/10
Hepatitis C Virus	20	5/20
Hepatitis B Surface Antigen	10	0/10
TOTALS	140	12 / 140

Twelve of the 140 samples tested yielded reactive results with the Immulite 2000 Syphilis Screen assay. These 12 samples were further tested with a commercially available blot assay and were verified as positive for the presence of antibody to *T. pallidum*.

f. Assay cut-off:

Test value = ratio of signal from sample to that of signal of adjustor curve parameter P1.

≥ 1.1 Reactive 0.9 to <1.1 Indeterminate <0.9 non reactive

2. Comparison studies:

a. Method Comparison with Predicate:

An in-house study was conducted in order to compare Immulite Syphilis Screen product to a commercially available Treponema Assay. A total of 280 samples from various patient populations were tested, with the results summarized in the following tables.

In-House Method Comparison, Lot 101

		Commercially Available Treponema Assay		Total
		Negative	Positive	
IMMULITE	Reactive	0	54	54
	Nonreactive	224	2	226
	Total	224	56	280

Table 18.7-2: Method Comparison Values

N	280	
Total Agreement	99.3%	278/280
Positive Agreement	96.4%	54/56
Negative Agreement	100.0%	224/224

There were two samples that were nonreactive with the Immulite 2000 assay but positive with the predicate assay. These samples were further tested with another Treponema Assay and both were negative by this assay.

b. Matrix comparison:

Alternate Sample Types

In order to assess the effect of alternate sample types, blood was collected from 88 volunteers into plain, heparin, EDTA and Becton Dickinson SST® Vacutainer tubes. Forty eight of 88 samples were spiked with antibodies positive to *T. pallidum* to obtain positive and cut-off levels. All samples were assayed by the Immulite 2000 Syphilis Screen assay. The results were subjected to linear regression analysis.

Table 18.5: Alternate Sample Types

Tube Type	Mean Ratio	n	Regression Equation	Correlation Coefficient (r)
Serum	3.29	88	Not Applicable	NA
SST	3.09	88	(SST) = 0.91 (Plain tubes) + 0.105	1.00
Heparin	3.65	88	(Heparin) = 1.06 (Serum) + 0.172	0.99
EDTA	2.76	88	(EDTA) = 0.78 (Serum) – 0.027	0.97

It was determined that although heparinized plasma may increase values at or near the cutoff level, heparinized plasma is an acceptable

sample type. EDTA plasma, however, is not recommended as a sample type and this is stated in the Limitations section of the package insert.

3. Clinical Studies:

a. *Clinical Sensitivity:*

Clinical studies were conducted at three external sites in California, Virginia and The Netherlands using the Immulite 2000 Syphilis Screen. A total of 1286 serum samples were collected and tested in the study. The study subjects ranged in age from < 18 years–65+ years, and 56% were male.

A method comparison study was performed comparing the IMMULITE 2000 Syphilis Screen method to results from a commercially available assay.

Medically Diagnosed Syphilis Patients				
	Liaison Treponema Assay			Total
Immolute Syphilis Assay	Equivocal	Negative	Positive	Total
Indeterminate	0	0	2	2
Non-Reactive	0	6	0	6
Reactive	1	2	270	273
Total	1	8	272	281

	95% Confidence Interval	
Positive Agreement:	270/272=99.3%	97.4% - 99.9%
Negative Agreement:	6/8=75%	34.9% - 96.8%
Overall Agreement:	276/281=98.2%	95.9% - 99.4%

Samples Sent for Routine Syphilis Testing				
	Liaison Treponema Assay			Total
Immolute Syphilis Assay	Equivocal	Negative	Positive	Total
Indeterminate	0	0	2	2
Non-Reactive	0	558	0	558
Reactive	0	5	359	364
Total	0	563	361	924

	95% Confidence Interval	
Positive Agreement:	359/361=99.4%	98.0% - 99.9%
Negative Agreement:	558/563=99.1%	97.9% - 99.7%
Overall Agreement:	917/924=99.2%	98.4% - 99.7%

HIV Positive Samples				
	Liaison Treponema Assay			Total
Immolute Syphilis Assay	Equivocal	Negative	Positive	Total
Indeterminate	0	0	1	1
Non-Reactive	0	152	0	152
Reactive	0	7	260	267
Total	0	159	261	420

	95% Confidence Interval	
Positive Agreement:	260/261=99.6%	97.9% - 100%
Negative Agreement:	152/159=95.6%	91.1% - 98.2%
Overall Agreement:	412/420=98.1%	96.3% - 99.2%

b. *Clinical Specificity*

See section M.3.a

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

Not applicable.

4. Clinical cut-off:

Not applicable

5. Expected Values/Reference Range:

Serum samples from 157 apparently healthy male and female volunteers were tested by the Immulite 2000 syphilis screen assay. These samples yielded a mean value of 0.12, a median value of 0.09 and a 95th percentile of 1.12. Results are expressed as a signal to cut off ratio

N. Proposed Labeling:

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

O. Conclusion:

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