

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K160910

B. Purpose for Submission:

To establish substantial equivalence to a predicate device and to obtain market clearance for a new assay designed to detect antibodies to *T. pallidum* in human serum and plasma.

C. Measurand:

Antibodies to *T. pallidum* (IgM and IgG)

D. Type of Test:

A qualitative double antigen sandwich electrochemiluminescence immunoassay for use on the cobas e 411 analyzer.

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Syphilis

G. Regulatory Information:

1. Regulation section:

866.3830

Treponema pallidum treponemal test reagents

2. Classification:

Class II

3. Product code:

LIP, enzyme linked immunoabsorption assay, *Treponema pallidum*

4. Panel:

H. Intended Use:

1. Intended use(s):

Assay:

Immunoassay for the *in vitro* qualitative detection of total antibodies (IgG and IgM) to *Treponema pallidum* in human serum and plasma. The test is intended as an aid in the diagnosis of syphilis infection in conjunction with clinical signs and symptoms.

The Elecsys Syphilis immunoassay is not intended for use in screening blood or tissue donors. The effectiveness of this assay in testing blood or tissue donors has not been established.

The electrochemiluminescence immunoassay “ECLIA” is intended for use on cobas e 411 analyzer.

Control:

PreciControl Syphilis is intended for the quality control of the Elecsys Syphilis immunoassay on cobas e 411 analyzer.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For Prescription Use

4. Special instrument requirements:

cobas e 411 analyzer

I. Device Description:

Assay

The Elecsys Syphilis immunoassay is a fully automated, qualitative assay that uses a double antigen sandwich format for the detection of IgM and IgG antibodies to *T. pallidum*. Recombinant *T. pallidum* antigens labeled with either biotin or a ruthenium complex bind to *T. pallidum*-specific IgG or IgM to form a double antigen sandwich complex. The sandwich complex binds to streptavidin-coated microparticles which can be immobilized magnetically to the surface of an electrode. Unbound substances are removed during a wash step using ProCell. A chemiluminescent substrate is then added to the reaction tube. Application of a

voltage to the electrode induces a chemiluminescent emission which is measured by a photomultiplier.

The presence or absence of anti-TP antibodies in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff index (COI) determined from an active calibration. The strength of the signal generated is proportional to the amount of bound enzyme and thus the amount of anti-*T. pallidum* antibodies present in the specimen. If the chemiluminescent signal in the reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-TP antibodies. If the chemiluminescent signal is below the cutoff signal, the specimen is considered nonreactive for the anti-TP antibodies.

The results are printed out as follows:

COI \geq 1.00	Reactive
COI < 1.00	Nonreactive

Interpretation of results:

Reactive	Reactive for treponemal antibodies
Nonreactive	Nonreactive for treponemal antibodies

Test results are intended to aid in diagnosis only. As with all serological tests for syphilis, results should always be interpreted in conjunction with additional treponemal or non-treponemal serologic test results (as appropriate), the patient's clinical symptoms, medical history, and other clinical and/or laboratory findings to produce a diagnosis of syphilis by disease stage.

All initially reactive samples should be retested in duplicate with the Elecsys Syphilis assay. If cutoff index values < 1.00 are found in both cases, the samples are considered negative for anti-*Treponema pallidum* antibodies.

Initially reactive samples with cutoff index values of \geq 1.00 in either of the retests are considered repeatedly reactive. Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms.

The PreciControl Syphilis 1 and 2 controls and Syphilis Cal1 and Cal2 calibrators are for use with the Elecsys Syphilis Assay.

Syphilis Cal1 and Cal2 Calibrators

Provided with the assay; intended for the calibration of the Elecsys Syphilis immunoassay. The calibrators are provided as two 1.0 mL bottles of lyophilized human serum each. Cal1 contains human serum which is non-reactive for anti-TP antibodies. Cal2 contains human serum which is reactive for anti-TP antibodies.

Elecsys Syphilis Assay Controls

The PreciControl Syphilis 1 and 2 controls are intended for the monitoring of accuracy for the Elecsys Syphilis assay and are sold as an accessory. The controls consist of two bottles of human serum positive or negative for anti-*Treponema pallidum* antibodies plus a preservative. Target values for the cutoff index are available electronically or provided in the control reagent kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Immulate 2000 Syphilis Screen Test

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

K091361

3. Comparison with predicate:

Characteristics	Elecsys Syphilis	Immulate 2000 Syphilis Screen Test System (K091361)
Similarities		
Intended Use	<p>Immunoassay for the <i>in vitro</i> qualitative detection of total antibodies (IgG and IgM) to <i>Treponema pallidum</i> in human serum and plasma. The test is intended as an aid in the diagnosis of syphilis infection in conjunction with clinical signs and symptoms.</p> <p>The Elecsys Syphilis immunoassay is not intended for use in screening blood or tissue donors. The effectiveness of this assay in testing blood or tissue donors has not been established.</p> <p>The electrochemiluminescence immunoassay “ECLIA” is intended for use on cobas e 411 analyzer.</p>	<p>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 on the IMMULITE 2000 analyzer as an aid I the diagnosis of syphilis.</p> <p>The IMMULITE 2000 Syphilis Screen test is not intended for use in screening blood or plasma donors.</p>
Analytes	Antibodies to <i>Treponema pallidum</i>	Same
Specimen Types	Serum and plasma	Same
Controls	2 (Negative and Positive)	Same
Differences		
Cut-off Index	<1.00 Non-reactive	<0.9 Non-reactive

	≥1.00 Reactive	≥0.9 to <1.1 Indeterminate ≥1.1 Reactive
Instrument	Cobas e 411	Immulite 2000 System
Antigens used	Recombinant antigens TpN17, TpN15, and TpN47	Recombinant antigen Tp17
Sample types	Serum, K ₂ EDTA, K ₃ EDTA, CPDA, NaCitrate and Li Heparin	Serum, heparinized plasma
Assay methodology	Double antigen sandwich, electrochemiluminescence immunoassay	Enzyme labeled, single step chemiluminescent immunoassay

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

Not applicable.

L. Test Principle:

Electrochemiluminescence Immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Internal Precision

The precision of the Elecsys Syphilis assay was evaluated in an internal study using six human serum samples (spiked), the PreciControl 1 and PreciControl 2 controls, and one lot of reagents. Two replicates of each serum sample and control were tested two times per day for 21 days. Samples were run in randomized order on the analyzer. Human serum samples were prepared to create a panel of two negative samples (COI < 1.0), two low positive samples (COI approximately = 1.0), and two positive samples (COI > 1.0). The mean COI value, within-run Standard Deviation (SD) and percent coefficient of variation (%CV) as well as between-run SD and %CV were calculated for each group of samples and a summary of the results is shown below.

Sample	N	Mean COI	Repeatability		Between Run	
			SD COI	%CV	SD COI	%CV
HS ¹ , negative 1	84	0.103	0.002	1.6	0.003	3.2
HS, negative 2	84	0.821	0.0174	2.1	0.019	2.3
HS, low positive 1	84	1.01	0.028	2.8	0.033	3.2
HS, low positive 2	84	1.12	0.018	1.6	0.022	1.9

Sample	N	Mean COI	Repeatability		Between Run	
			SD COI	%CV	SD COI	%CV
HS, positive 3	84	9.99	0.171	1.7	0.262	2.6
HS, positive 4	84	50.2	0.986	2.0	1.24	2.5
PreciControl Syphilis1	84	0.106	0.003	2.4	0.004	4.1
PreciControl Syphilis2	84	4.95	0.101	2.1	0.161	3.2

1) HS = human serum

Multi-site Reproducibility

A reproducibility study was conducted following CLSI EP5-A2 at three sites incorporating a seven member panel consisting of five serum pools: high negative (COI <1.0; HSP 08 and HSP09), low positive (COI close to 1.0; HSP 06 and HSP 07) and moderate positive (COI >1.0; HSP 10). The five serum pools and two controls were assayed for five days, two runs per day, and three replicates per sample. Data from all three sites were combined to examine the standard deviation and percent coefficient of variance for repeatability within-run, between run, between-day, between site, and overall reproducibility. Data are summarized in the tables below.

Sample	N	Mean	Within Run Repeatability			Between Run	
		COI ¹	SD ²	95% CI	%CV	SD	%CV
HSP ³ 06	90	1.02	0.01	(0.01, 0.01)	1.16	0.02	2.28
HSP 07	90	1.13	0.02	(0.02, 0.03)	1.90	0.03	2.57
HSP 08	90	0.94	0.01	(0.01, 0.02)	1.58	0.02	2.55
HSP 09	90	0.85	0.02	(0.01, 0.02)	1.88	0.02	2.49
HSP 10	90	3.24	0.09	(0.08, 0.11)	2.78	0.08	2.61
SYPH PC1 ⁴	90	0.12	0.00	(0.00, 0.00)	2.41	0.00	2.39
SYPH PC2	90	4.76	0.08	(0.07, 0.10)	1.65	0.14	2.89

		Mean	Between Day		Between Site		Overall Reproducibility		
Sample	N	COI	SD	%CV	SD	%CV	SD	95% CI	%CV
HSP 06	90	1.02	0.00	0.00	0.01	1.29	0.03	(0.02, 0.04)	2.86
HSP 07	90	1.13	0.00	0.00	0.01	0.79	0.04	(0.03, 0.05)	3.29
HSP 08	90	0.94	0.00	0.00	0.01	0.75	0.03	(0.02, 0.04)	3.09
HSP 09	90	0.85	0.00	0.00	0.01	1.23	0.03	(0.02, 0.04)	3.36
HSP 10	90	3.24	0.00	0.00	0.00	0.00	0.12	(0.11, 0.15)	3.82
SYPH PC1	90	0.12	0.00	1.15	0.01	4.47	0.01	(0.00, 0.02)	5.73
SYPH PC2	90	4.76	0.00	0.00	0.03	0.70	0.16	(0.13, 0.21)	3.40

¹COI = cutoff index

²SD = standard deviation

³HSP = Human serum pool

⁴PC1/PC2 = PreciControl 1 / PreciControl 2

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay.

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

Calibrator

Calibrators are provided with the assay and are intended for the calibration of the Elecsys Syphilis immunoassay. The calibrator is referenced to a Roche internal reference standard. This internal reference standard is manufactured by dilution of high titer positive serum with nonreactive human serum.

External Controls

The Elecsys Syphilis immunoassay controls are sold as an accessory to the test. The Negative Control is made from human serum shown to be non-reactive for antibodies to TP. The Positive Control is made from TP-non-reactive human serum spiked with high titer anti-TP reactive human serum. During the clinical study, the positive control was analyzed 270 times; the mean COI was 4.63 with a SD of 0.207.

Sample Stability Studies

The stability of TP antibodies in serum, K₂-EDTA, K₃-EDTA, Li-Heparin, and CPDA plasma/Sodium-Citrate plasma was evaluated using eight different spiked samples (negative human serum spiked with varying levels of anti-TP antibodies). Reference samples, in their respective matrix, were stored at -80°C for the duration of the experiment and then thawed and tested along with paired test samples for each storage condition. Recovery for samples stored under the test condition was determined by

comparing the COI of the reference samples to the test samples. Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

Of the eight samples tested for each condition, three samples were prepared just below the cutoff for reactivity (COI < 1.0), three samples near the cutoff (COI = 1.0) and two samples were tested above the cutoff (COI > 1.0). All samples were tested in triplicate for a total of 24 tests per storage condition.

The stability of TP antibodies in various storage matrices was evaluated for:

- Refrigerated stability (at 2 to 8°C) for 21 days
- Room temperature stability (at 15 to 25°C) for 8 days
- Frozen stability (at -15 to -25°C) for 12 months
- Freeze/thaw stability after 5 cycles (a freeze/thaw cycle was defined as frozen at \leq -10°C for > 12 hours)

All tests met the acceptance criteria for all the anti-coagulants and storage conditions tested. Roche is claiming the following storage conditions for specimens:

Serum, K₂-EDTA, K₃-EDTA, Li-Heparin, CPDA plasma, Sodium-Citrate plasma at:

- 2 to 8°C for 14 days
- 15 to 25°C for 5 days
- -15 to -25°C for 12 months
- Samples may be frozen and thawed up to 5 times

Reagent Stability

To test reagent stability, a fresh reagent Rack-Pack was placed on the analyzer and calibrated. Reference values were determined and the kit was removed from the analyzer and stored at 2 to 8°C for 56 days. On days 28 and 56 the kit was placed on the analyzer, calibrated and samples were tested again. Eight spiked samples plus a positive and negative control were tested in duplicate both for the reference point and days 28 and 56. Of the eight samples tested, three samples were prepared just below the cutoff for reactivity (COI < 1.0), three samples near the cutoff (COI = 1.0) and two samples were tested above the cutoff (COI > 1.0). Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

All samples tested met the acceptance criteria. Roche has claimed reagent stability up to day 56 after opening a reagent Rack-Pack when the pack is kept at 2 to 8°C.

On-board Reagent Stability

The stability of Elecsys Syphilis reagent Rack-Pack left on the instrument was evaluated on days 0, 1, 8, 15, 22, and 30. Day 0 was used as the reference point to determine COI deviation. Eleven human serum samples plus two controls were evaluated on each day using the same reagents kept at 20 °C on-board the instrument. Calibration curves established for the previous time-point were used for days 8, 15, 22, and 30. Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

All samples tested met the acceptance criteria. Roche has claimed 28 days in the package insert for on-board reagent stability.

Shelf Life

The control reagent was measured after 7, 9, 13, 16, and 19 months storage at 2 to 8°C. Studies were performed with three lots of stored reagents and tested in duplicate for each lot. Percent recovery was calculated based on the target value of the Control Reagent. Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

The data demonstrated reagent stability for at least 18 months when stored at 2 to 8°C. Roche has indicated a 15 month shelf life for the control reagents.

Calibrator Stability

Roche performed a calibration study using spiked human serum samples and fresh reagent kits on a single analyzer calibrated with a single lot of reagents. Recovery (% COI) was determined on days 28 and 56 and compared to the results obtained on day 0. Eight human serum samples plus controls were tested in duplicate. Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

All samples met the acceptance criteria on days 28 and 56. Roche is claiming a Calibrator stability of 28 days in the package insert.

On-Board Calibrator Stability

The stability of Elecsys Syphilis calibrator left on the instrument was evaluated on days 0 and 8. Day 0 was used as the reference point to determine COI deviation. Eleven human

serum samples plus two controls were evaluated in duplicate using the same reagents kept at 20 °C on-board the instrument. The calibration curve established on day 0 was used for the day 8 testing.

Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

All samples tested met the acceptance criteria. Roche has claimed seven days in the package insert for on-board calibrator stability.

Hook Effect

The sponsor conducted a study to demonstrate that the Elecsys Syphilis immunoassay does not produce false non-reactive results when testing samples with high levels of anti-TP antibody. The study utilized six samples with medium to high levels of TP-antibody which were diluted with negative (TP-Ab non-reactive) serum. Each of the diluted samples was tested with the Elecsys Syphilis assay. Total RFU counts were plotted for each sample as a function of dilution level. Acceptance criterion set by Roche described the data as acceptable if there were no samples which resulted in a COI less than 3 due to high antibody concentration. The COI of undiluted serum samples ranged from 276 to 345.

The acceptance criteria were met in all samples tested. Plots of each sample dilution series supported the conclusion that there was no high dose hook effect. These data demonstrate that the Elecsys Syphilis assay is not susceptible to interference from specimens with high levels of anti-syphilis TP.

Carryover

Previous carryover studies performed on the same instrument demonstrated the absence of carry-over on the cobas e 411 analyzer for other Elecsys assays. In addition, cobas e 411 analyzer utilizes disposable materials to reduce the probability of contamination due to carryover.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Interference by Endogenous Substances

Interference by endogenous substances was evaluated by testing three anti-*T. pallidum* antibody concentrations (negative, near cut-off and positive). Negative sample pools were prepared with an expected COI value between 0 and 1. Near cut-

off sample pools were prepared such that the expected COI value was between 1.0 and 2.0. Positive sample pools were prepared with an expected COI of greater than 5.0. Each serum pool was spiked with the interferent at varying concentrations up to the maximum concentrations indicated in the table below. A total of 10 samples (ten 10-fold dilutions) were tested for each serum pool for each potential interferent. Recovery was calculated for the COI of each sample as the percent of the expected result (sample with no interferent present). Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 15% COI

Potential Interferent	Substance Measured up to	No Interference Seen up to	Label Claim
Intralipid (Lipemia)	2000 mg/dL	2000 mg/dL	2000 mg/dL
Biotin	70 ng/mL	70 ng/mL	60 ng/mL
Bilirubin	66 mg/dL	66 mg/dL	66 mg/dL
Hemoglobin	1000 mg/dL	1000 mg/dL	500 mg/dL
Rheumatoid Factor	1500 IU/mL	1500 IU/mL	1500 IU/mL
Human Serum Albumin	10 g/dL	10 g/dL	10 g/dL
Human IgG	7.0 g/dL	6.3 g/dL	32 g/L
Human IgM	1.0 g/dL	1.0 g/dL	10 g/L
Human IgA	7.0 g/dL	2.8 g/dL	2.8 g/dL

Drug Interference

Potential interference due to the presence of common pharmaceutical compounds was tested in a drug interference study. Human serum samples were spiked with anti-TP antibodies at high negative (COI <1.0) or at low positive levels (COI <2.0). These serum samples were tested in the presence or absence of sixteen commonly used pharmaceuticals. Absolute COI (for high negative samples) and % COI (for low positive samples) were compared to the reference COI to determine whether interference occurred. A list of the drugs and concentrations tested are shown in the table below. No interference was observed at the levels tested.

Drug Tested	Concentrations Tested
Acetylcystein	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic Acid	300 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U/L
Levodopa	20 mg/L
Methyldopa + 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Doxycyclin	50 mg/L
Acetylsalicylic Acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L

Cross-reactivity in Specimens with Medical Conditions not Related to Syphilis

Specimens from individuals diagnosed with other diseases were obtained from commercial vendors or were sourced from leftover clinical specimens. The presence of the given analyte (microorganism or antibody) was confirmed with an FDA-cleared assay. The specimens were tested by the Elecsys Syphilis assay. Positive samples were also tested by the BioRad Syphilis EIA II Total Antibody Assay. The results of the specificity study in samples from individuals with other diseases are shown below.

Potential Cross-reactant	Number Tested	Number of Positive Samples
<i>Borrelia</i>	50	0
EBV IgG	21	1
Rubella IgG	17	1
<i>E.coli</i> antibodies	18	1
Hepatitis A	25	1
Hepatitis B	10	2
Hepatitis C	13	0
ANA ¹	15	1
RF ²	19	0
HSV 1/2	12	1
CMV IgG	20	0
HIV-1	25	1
<i>Toxoplasma</i> IgG	21	0
Total	266	9

¹ Anti-nuclear antigen

² Rheumatoid factor

The nine samples that were reactive with the Elecsys Syphilis assay were confirmed positive for anti-treponemal antibodies with the BioRad Syphilis EIA II Total Antibody Assay. The Elecsys Syphilis assay does not appear to cross-react with any of the analytes tested above.

f. Assay cut-off:

In order to define the cut-off for the Elecsys Syphilis assay, native human serum samples were measured with a prototype lot of the Elecsys Syphilis assay and a preliminary cut-off was set. Next, the Elecsys syphilis results from patient samples were compared to the results from other commercially available assays. The results of the subsequent multi-center clinical studies conducted in the EU and the US provided final validation for the cut-off value.

Result classification:

Sample result < 1.0 COI sample is interpreted as “non-reactive”

Sample result ≥ 1.0 COI sample is interpreted as “reactive”

2. Comparison studies:

a. Method comparison with predicate device:

The results of the Elecsys Syphilis assay were compared to a composite comparator based on an algorithm of results obtained from three commercially available syphilis assays: (a) a treponemal chemiluminescent immunoassay (Predicate), a non-treponemal assay (Rapid Plasma Reagin [RPR]), and a second treponemal assay (*Treponema Pallidum* Particle Agglutination [TP-PA]). Additional details of how the final comparator result was determined are provided below in the “Clinical Studies” section.

b. Matrix comparison:

The effect of anticoagulants in the samples on the performance of the Elecsys Syphilis Immunoassay was determined by comparing values obtained from native samples spiked with anti-*T. pallidum* antibodies (single donors) drawn into the following vacutainer tube types:

- Serum (plastic)-Used as the reference
- Serum separator tubes (SST)
- CPDA
- Dipotassium-EDTA plasma
- Tripotassium-EDTA plasma
- Lithium-Heparin plasma
- Sodium-Citrate
- Dipotassium-EDTA plasma preparation tube (PPT)

A total of 57 serum/plasma pairs were tested for each anti-coagulant tube. The matrices were spiked with anti-*T. pallidum* antibodies to produce samples ranging from below the COI cutoff (COI < 1.0) to high positive (COI > 10). The panel also included many samples near the cutoff (COI close to 1.0). Recovery of the analyte as measured by COI or % COI in the presence of different anticoagulants was determined. Acceptance criteria for recovery were:

- For reference COI < 1.0 deviation \pm 0.2 COI
- For reference COI > 1.0 deviation \pm 20% COI

Matrix	Sample Size	Range	Maximum Deviation from Reference (%)	Maximum Absolute Deviation from Reference (COI)
Serum	57	0.130-62.8	N/A	N/A
SST	57	0.130-60.9	16%	0.030
K ₂ -EDTA plasma	57	0.130-66	9%	0.040
K ₃ -EDTA plasma	57	0.130-66.3	12%	0.050

Li-Heparin plasma	57	0.120-51.9	15%	0.070
CPDA	57	0.130-55.3	7%	0.120
Na-Citrate	57	0.120-65.8	5%	0.090
K ₂ -EDTA (PPT)	57	0.130-70	12%	0.070

The specifications were met for all anticoagulants. The resulting data support the package insert claim that serum, serum separation tubes, Li-Heparin plasma, K₂-EDTA- and K₃-EDTA-plasma, Na–citrate, CPDA plasma and K₂-EDTA (PPT) specimens are acceptable for use with the Elecsys Syphilis Immunoassay.

3. Clinical Studies:

A multicenter study was conducted from June 2014 to December 2014 (5 sites) and from August 2015 to November 2015 (4 sites) to evaluate the ability of the Elecsys Syphilis assay to detect antibodies (IgG and IgM) directed against *Treponema pallidum* (TP). A total of 2697 subjects were involved in the clinical study of which 37 did not meet the inclusion/exclusion criteria. The remaining 2660 specimens were tested in the Elecsys Syphilis Immunoassay clinical study. There were no samples excluded from the study after enrollment. The 2660 specimens consisted of 2282 specimens obtained through routine syphilis testing, 169 pre-selected samples presumed to be positive for antibodies directed against TP based on previous laboratory testing (including 15 pregnant women known to be reactive for syphilis antibodies), and 209 specimens from apparently healthy individuals. An additional 40 specimens from pregnant females negative for *T. pallidum* antibodies and spiked with individual high antibody-positive specimens were also tested in a separate study.

The 2660 specimens analyzed in the Elecsys Syphilis Immunoassay clinical study were collected at clinical sites in the following locations:

- Miami, Florida: 6.84%
- Spartanburg, North Carolina: 26.28%
- Baltimore, Maryland: 21.95%
- Minneapolis, Minnesota: 0.98%
- Whittier, California: 29.14%
- Miami Beach, Florida: 0.41%
- Tamarac, Florida: 8.20%
- Buenos Aires, Argentina: 5.64%
- Nanikon, Switzerland: 0.56%

The clinical performance of the Elecsys Syphilis assay was evaluated by calculating positive percent agreement and negative percent agreement of the assay with the final comparator result based on an algorithm of results from three commercially available syphilis assays: an FDA-cleared treponemal chemiluminescent immunoassay

(“Predicate”), a non-treponemal assay (Rapid Plasma Reagin [RPR]), and a second treponemal assay (*Treponema Pallidum* Particle Agglutination [TPPA]. Because the clinical diagnosis of syphilis must be supported by two reactive laboratory tests, consisting of a treponemal assay and a non-treponemal assay, or at least two treponemal assays, employing an algorithm of three syphilis tests to determine the comparator result presents information closest to the serological “truth.”

The final comparator result was determined using a two out of three rule (Predicate, RPR, and TPPA). The table below shows how the final comparator result was interpreted for the Elecsys Syphilis assay clinical study.

Treponemal (Predicate)	Non-treponemal (RPR)	2nd Treponemal (TPPA)	Final Comparator Result
Negative	Negative	Positive	Negative
		Negative	Negative
		Inconclusive	Negative
Negative	Positive	Positive	Positive
		Negative	Negative
		Inconclusive	Negative
Positive	Positive	Positive	Positive
		Negative	Positive
		Inconclusive	Positive
Positive	Negative	Positive	Positive
		Negative	Negative
		Inconclusive	Negative
Indeterminate	Negative	Positive	Positive
		Negative	Negative
		Inconclusive	Indeterminate
Indeterminate	Positive	Positive	Positive
		Negative	Negative
		Inconclusive	Indeterminate

Clinical Performance in Prospectively Collected Specimens in the Intended Use Population

The 2282 prospectively collected specimens in the intended use population, analyzed in the Elecsys Syphilis assay clinical study consisted of 1524 specimens sent for routine syphilis testing (60% female, 40% male, 18-79 years old), 301 pregnant females (18–45 years old), and 457 HIV positive individuals (40% female, 60% male, 18–70 years old). Each of those specimens was analyzed with the Elecsys Syphilis assay and with the three reference assays.

A summary of the serological test profile for all prospectively-collected specimens in the intended use population is presented in the following table.

Predicate Result	RPR	TPPA	Final Comparator Result	Elecsys Syphilis	Number of Subjects
NR ¹	NR	N/A ²	Negative	NR	2023
NR	NR	Inconclusive	Negative	NR	1
NR	NR	NR	Negative	NR	1
NR	NR	NR	Negative	Reactive	1
NR	NR	Reactive	Negative	Reactive	4
NR	Reactive	NR	Negative	NR	4
Reactive	NR	Inconclusive	Negative	Reactive	2
Reactive	NR	NR	Negative	NR	9
Reactive	NR	NR	Negative	Reactive	9
Reactive	NR	Reactive	Positive	Reactive	166
Reactive	Reactive	N/A	Positive	Reactive	62

¹ NR = Non-reactive

² N/A = Test not performed

The comparison between the Elecsys Syphilis assay result and the final comparator result for the prospectively-collected specimens in the intended use population is shown in the following table.

Percent Agreement in Prospective Specimens

	Final Comparator Results		
Elecsys Syphilis	Positive for Syphilis	Negative for Syphilis	Total
Reactive	228	16	244
Non reactive	0	2038	2038
Total	228	2054	2282

Positive percent agreement for the Elecsys Syphilis assay in the prospective cohort was 100% (228/228) with a 95% confidence interval of 98.4% to 100.0%. Negative percent agreement was 99.2% (2038/2054) with a 95% confidence interval of 98.7% to 99.6%.

Percent Agreement by Category for Prospective Specimens

Cohort	Positive Percent Agreement		PPA 95 % CI	Negative Percent Agreement		NPA 95 % CI
	%	Ratio		%	Ratio	
Routine Syphilis	100	66/66	94.56 to 100.00	99.8	1455/1458	99.40 to 99.96
HIV	100	162/162	97.75 to 100.00	95.6	282/295	92.58 to 97.63
Pregnant	N/A	0/0	N/A	100	301/301	98.78 to 100.00
Total	100	228/228	98.40 to 100.00	99.2	2038/2054	98.74 to 99.55

Clinical Performance in Retrospective Samples (Pre-selected)

Clinical performance in the pre-selected retrospective cohort was evaluated by testing a total of 169 specimens, including 15 pregnant positive women and 154 subjects medically diagnosed with syphilis at different stages. The comparison between the Elecsys Syphilis results and the final comparator results is shown in the following table.

Elecsys Syphilis	Final Comparator Results		Total
	Positive for Syphilis	Negative for Syphilis	
Reactive	155	0	155
Non-reactive	2	12	14
Total	157	12	169

Positive percent agreement for the Elecsys Syphilis assay in the retrospective cohort was 98.7% (155/157) with a 95% confidence interval of 95.5% to 99.85%. Negative percent agreement was 100.0% (12/12) with a 95% confidence interval of 73.5% to 100.0%

Percent Agreement by Category for Retrospective Specimens

Category	Positive Percent Agreement		PPA 95 % CI	Negative Percent Agreement		NPA 95 % CI
	%	Ratio		%	Ratio	
Pregnant (Retrospective)	100	15/15	78.20 to 100.00	N/A	0/0	N/A
Staged	98.6	140/142	95.00 to 99.83	100	12/12	73.54 to 100.00
Overall (Retrospective)	98.7	155/157	95.47 to 99.85	100	12/12	73.54 to 99.55

Clinical Performance in Medically Diagnosed Individuals

Samples were collected from 154 individuals diagnosed with primary, secondary or latent syphilis. They included 10 females and 144 males. Results of the Elecsys Syphilis assay for this cohort are summarized below.

Reactivity of the Elecsys Syphilis Assay in Subjects Medically Diagnosed with Syphilis

Medically Diagnosed Individuals			Elecsys Syphilis Result	
Syphilis Stage	Treatment Status	N	Reactive	Nonreactive
Primary	Treated	29	16	13*
	Untreated	25	25	0
Secondary	Treated	25	24	1
	Untreated	25	25	0
Latent	Treated	25	25	0
	Untreated	25	25	0

* 12 of these samples also tested negative for Syphilis with the composite testing algorithm

Clinical Performance in Pregnant Females

A total of 316 pregnant female samples were tested in the study. Of these, 301 were prospectively collected and 15 were retrospectively collected. The percent agreement between the Elecsys Syphilis results and the final comparator results is shown below, stratified by pregnancy trimesters compared with the Composite Algorithm for pregnant women.

Percent Agreement in Pregnant Women

Category	PPA		PPA 95% CI	NPA		NPA 95% CI
	%	Ratio		%	Ratio	
Pregnant (Prospective)	N/A	0/0	N/A	100	301/301	98.8-100.0
1 st Trimester	N/A	0/0	N/A	100	100/100	96.4-100.0
2 nd Trimester	N/A	0/0	N/A	100	125/125	97.1-100.0
3 rd Trimester	N/A	0/0	N/A	100	76/76	95.3-100.0
Pregnant (Retrospective)	100	15/15	78.2–100.0	N/A	0/0	N/A
1 st Trimester	100	7/7	59.0-100.0	N/A	0/0	N/A
3 rd Trimester	100	8/8	63.1-100.0	N/A	0/0	N/A

In addition, 40 specimens from nonreactive pregnant females, spiked with samples known to be highly reactive for TP antibodies, were analyzed with the Elecsys Syphilis assay. All samples tested reactive.

Clinical Performance in Apparently Healthy Individuals

Specimens were collected from 209 apparently healthy individuals. Of these, 80 were female and 129 male. The results of the Elecsys Syphilis assay for this group are shown below.

	Elecsys Syphilis Result		
	# Reactive	# Non-reactive	
Female	9 (11.3%)	71 (88.7%)	80
Male	11 (8.5%)	118 (91.5%)	129
Total	20 (9.6%)	189 (90.4%)	209

a. Clinical Sensitivity:

The clinical sensitivity of the assay was expressed as percent agreement of the Elecsys Syphilis assay results with the final comparator result, as explained above.

b. Clinical specificity:

The clinical specificity of the assay was expressed as percent agreement of the Elecsys Syphilis assay results with the final comparator result, as explained above.

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

There were no invalid results recorded by the Elecsys Syphilis Immunoassay during the clinical study.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

In this clinical study there were 2282 prospectively collected specimens for the intended use population that were tested with the Elecsys Syphilis assay. There were 244 reactive samples for a 10.7% prevalence of *T. pallidum* antibodies in the study population. The distribution of the Elecsys Syphilis reactive and non-reactive results is summarized below by age and gender.

		Elecsys Syphilis Results		
Age Range (years)	Gender	Reactive	Non-reactive	Total
18-21	Female	0 (0.00%)	247 (100%)	247
	Male	6 (3.5%)	165 (96.5%)	171
22-29	Female	0 (0.00%)	358 (100%)	358
	Male	19 (10.7%)	159 (89.3%)	178
30-39	Female	10 (3.7%)	261 (96.3%)	271
	Male	13 (12.3%)	93 (87.7%)	106
40-49	Female	29 (12.1%)	210 (87.9%)	239
	Male	29 (20.0%)	116 (80.0%)	145
50-59	Female	47 (20.8%)	179 (79.2%)	226
	Male	55 (27.1%)	148 (72.9%)	203
60-69	Female	11 (23.9%)	35 (76.1%)	46
	Male	23 (29.5%)	55 (70.5%)	78
70-79	Female	0 (0.00%)	5 (100%)	5
	Male	2 (22.2%)	7 (77.8%)	9
Combined	Total	244 (10.7%)	2038 (89.3%)	2282

N. Instrument Name:

cobas e 411 Analyzer

O. System Descriptions:

The Elecsys Syphilis Immunoassay is performed on the cobas e 411 analyzer using previously cleared software. The software version has been updated to include the Elecsys Syphilis test to the test menu.

1. Modes of Operation:

This is a fully automated immunoassay analyzer.

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes or No

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes or No

2. Software:

The latest Elecsys Syphilis Immunoassay is performed on the cobas e 411 analyzer using software version 02-04.

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

Yes or No

3. Specimen Identification:

The specimens are identified by an internal barcode reader.

4. Specimen Sampling and Handling:

The specimen processing is automated. The operator only needs to place the primary sample tube in the instrument sample carrier (disk or rack systems).

5. Calibration:

Assays do not need to be calibrated every time they are run; however, certain variables make recalibration necessary. Calibration must be performed when a new reagent lot is used, when documentation accompanying a new version of an existing assay states that calibration is required, and when the controls are out of range.

6. Quality Control:

Quality control is performed using the Elecs PreciControl Syphilis Cal1 and Syphilis Cal2. The positive control (inactivated) is reactive for anti-TP. Controls are not barcode labeled and need to be run like external controls.

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.