

Elecsys Syphilis

REF			SYSTEM
09014977190	09014977501	100	cobas e 411 cobas e 601 cobas e 602

English

For use in the USA only

System information

For **cobas e 411** analyzer: test number 2370

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 149

Intended use

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 a nontreponemal laboratory test and 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** immunoassay analyzers.

Summary

Syphilis is caused by the intracellular gram-negative spirochete bacterium *Treponema pallidum* (TP) subspecies *pallidum*.¹

Syphilis is mainly transmitted sexually, but also can be transmitted from mother to fetus during pregnancy or birth. Based on prevalence data from 2009 to 2016, the estimated global prevalence of syphilis in both men and women was 0.5 %, with regional values ranging from 0.1 to 1.6 %, corresponding to 19.9 million syphilis cases.² In 2020, WHO estimated 7.1 million new syphilis infections globally.³ In the US, 133945 cases of all stages of syphilis were reported in 2020. Since reaching a historic low in 2000 and 2001, the rate of primary and secondary syphilis has increased almost every year, increasing 6.8 % during 2019-2020.⁴ Certain European countries have also seen increases in the rate of infection and large localized outbreaks.⁵

Congenital syphilis in the newborn is still common in the developing world, as many women do not receive prenatal care or the care does not include syphilis screening.⁶ The estimated global maternal syphilis prevalence in 2016 was 0.69 %, resulting in 661000 total congenital syphilis cases, including 355000 adverse birth outcomes and 306000 non-clinical congenital syphilis cases (infants without clinical signs born to untreated mothers).⁷ WHO and US Preventive Services Task Force recommend that all women be tested at their first prenatal visit and again in the third trimester.^{8,9} If they are positive, sexual partners should be evaluated and offered treatment.¹⁰ Syphilis infection facilitates HIV infection.¹¹

In the early stage of infection, the clinical diagnosis of syphilis can be very difficult.¹ Typically, symptoms of syphilis start with a painless ulcer at the site of entry to the body (primary syphilis) followed by a widespread rash as the bacteria disseminate (secondary syphilis). This is followed by a lengthy latent (asymptomatic) period. Eventually, tertiary syphilis ensues, characterized by the development of granulomatous dermal lesions, neurosyphilis, and/or cardiovascular syphilis (which can be fatal).¹² The immune response to *T. pallidum* is the main driver of lesion development.¹² The antibody response is directed not only against antigens specific to *T. pallidum* (treponemal antibodies), but antibodies are also generated against antigens which are not specific (non-treponemal antibodies); for example, antigens released during the cellular damage caused by the organism. Therefore, treponemal and non-treponemal tests co-exist for the diagnosis of syphilis.¹

Non-treponemal tests use antigens comprising lecithin, cholesterol, and purified cardiolipin to detect antibodies against cardiolipin, which are present in many syphilis patients. Treponemal tests detect antibodies directed against *T. pallidum* proteins. A positive treponemal antibody test result indicates prior exposure to syphilis. Non-treponemal assays are useful for monitoring the progression of disease and response to therapy. Both tests are necessary as an aid of the diagnosis.¹

The Elecsys Syphilis assay uses recombinant antigens representing the lipoproteins TpN17, TpN15 and TpN47 for the detection of anti-*Treponema pallidum* antibodies.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, biotinylated TP-specific recombinant antigens and TP-specific recombinant antigens labeled with a ruthenium complex^{a)} react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as Syphilis.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 TP-specific recombinant antigens (*E. coli*)-biotin (gray cap), 1 bottle, 9 mL: Biotinylated TP-specific recombinant antigens (*E. coli*) 0.7 mg/L; MES^{b)} buffer 50 mmol/L, pH 6.5; preservative.
- R2 TP-specific recombinant antigens (*E. coli*)-Ru(bpy)₃²⁺ (black cap), 1 bottle, 9 mL: TP-specific recombinant antigens labeled with ruthenium complex 0.7 mg/L; MES buffer 50 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

- Syphilis Cal1 Negative calibrator (white cap), 2 bottles (lyophilized) for 1.0 mL each: Human serum, non-reactive for anti-TP antibodies; preservative.
- Syphilis Cal2 Positive calibrator (black cap), 2 bottles (lyophilized) for 1.0 mL each: Human serum, reactive for anti-TP antibodies; preservative.

Precautions and warnings

For in vitro diagnostic use for healthcare professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Warning

H317 May cause an allergic skin reaction.

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H412 Harmful to aquatic life with long lasting effects.

Prevention:

P261 Avoid breathing mist or vapours.

P273 Avoid release to the environment.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride
- Product safety labeling follows EU GHS guidance.
Contact phone: 1-800-428-2336

All human material should be prepared potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{13,14}

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M1, R1, R2) in the kit are ready for use and are supplied in bottles compatible with the system.

Calibrators:

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

cobas e 411 analyzer: The reconstituted calibrators should only be left on the analyzers during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

If necessary, freeze in aliquots; see section on **cobas e 601** and **cobas e 602** analyzers.

cobas e 601 and **cobas e 602** analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the reconstituted calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at -20 °C (± 5 °C) for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note for **cobas e 602** analyzers: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. Please turn the vial cap 180° into the correct position so that the barcode between the yellow markers can be read by the system. Place the vial on the analyzer as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

<i>Stability of the reagent rackpack</i>	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	56 days
on the analyzers	28 days

The lyophilized calibrators are stable up to the stated expiration date.

<i>Stability of the reconstituted calibrators</i>	
either at -20 °C (± 5 °C)	6 months (3 freeze/thaw cycles possible)
or at 2-8 °C	28 days
on cobas e 411 analyzer at 20-25 °C	up to 6 hours
on cobas e 601 and cobas e 602 analyzers at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K₂-EDTA, K₃-EDTA, CPDA and Na-citrate plasma.

Li-heparin plasma tubes containing separating gel can be used.

Criterion: Mean recovery of positive samples within ± 20 % of serum value. Absolute deviation of samples with COI values from 0.00-1.0 within ± 0.2 COI.

Sampling devices containing liquid anticoagulants have a dilution effect resulting in lower COI values for individual patient specimens. In order to minimize dilution effects it is essential that respective sampling devices are filled completely according to manufacturer's instructions.

Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 12 months at -20 °C (± 5 °C). The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube/collection system manufacturer.

Centrifuge samples containing precipitates and frozen samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- [\[REF\]](#) 06923364190, PreciControl Syphilis, for 4 x 2.0 mL

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- [REF] 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** analyzer
- Distilled or deionized water

Additional materials for **cobas e** 411 analyzer:

- [REF] 11662988122, ProCell, 6 x 380 mL system buffer
- [REF] 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- [REF] 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- [REF] 04880340190, ProCell M, 2 x 2 L system buffer
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF] 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- [REF] 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- [REF] 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the reconstituted calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (**cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against a Roche standard. The units have been selected arbitrarily.

Calibration frequency: Calibration must be performed once per reagent lot using Syphilis Cal1, Syphilis Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- every 28 days when using the same reagent lot
- every 7 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings with PreciControl Syphilis outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (Syphilis Cal1): 450-4000,
Positive calibrator (Syphilis Cal2): 22000-140000.

Quality control

Use PreciControl Syphilis or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of Syphilis Cal1 and Syphilis Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff).

Interpretation of the results

Samples with a cutoff index < 1.00 are non-reactive in the Elecsys Syphilis assay. These samples are considered negative for anti-*Treponema pallidum* antibodies.

Samples with a cutoff index ≥ 1.00 are considered reactive in the Elecsys Syphilis assay.

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 giving cutoff index values of ≥ 1.00 in either of the retests are considered repeatedly reactive. Repeatedly reactive samples must be supplemented according to recommended confirmatory algorithms. Reactive treponemal test results alone are not diagnostic of syphilis, treponemal test results should always be interpreted in conjunction with additional treponemal or nontreponemal serologic test results (as appropriate), medical history, clinical presentation and other findings, as recommended by the Centers for Disease Control and Prevention (CDC) Sexually Transmitted Diseases Treatment Guidelines.

Limitations

No false negative result due to high-dose hook effect was found with the Elecsys Syphilis assay.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

The detection of treponemal antibodies may indicate recent, past or successfully treated syphilis. This test cannot distinguish between active and treated infection, and therefore, may not be used to determine response to therapy, relapse, or reinfection.

A negative test result does not completely rule out the possibility of an infection with *Treponema pallidum*. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of a syphilis infection can occasionally yield negative findings.

Assay interference due to circulating antibodies against *Treponema pallidum* subspecies causing yaws, pinta and bejel has not been evaluated. Cross-reactivity with these antibodies is to be expected.

Analytical performance

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean COI ^{c)}	SD COI	CV %	SD COI	CV %
HS ^{d)} , negative 1	0.145	0.001	0.9	0.006	3.9
HS, negative 2	0.894	0.019	2.1	0.037	4.1
HS, positive 1	1.06	0.027	2.5	0.066	6.3
HS, positive 2	3.77	0.101	2.7	0.251	6.7
HS, positive 3	6.80	0.238	3.5	0.435	6.4
HS, positive 4	15.3	0.499	3.3	0.877	5.7
PreciControl Syphilis1	0.123	0.002	1.6	0.004	3.3
PreciControl Syphilis2	5.80	0.142	2.4	0.260	4.5

c) Cutoff Index

d) HS = human serum

cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS, negative 1	0.100	0.001	1.3	0.006	5.7
HS, negative 2	0.877	0.021	2.4	0.041	4.6
HS, positive 1	1.09	0.016	1.5	0.058	5.4
HS, positive 2	3.99	0.095	2.4	0.249	6.3
HS, positive 3	6.87	0.151	2.2	0.326	4.7
HS, positive 4	16.0	0.282	1.8	0.697	4.4
PreciControl Syphilis1	0.076	0.001	1.5	0.003	4.4
PreciControl Syphilis2	6.01	0.133	2.2	0.255	4.2

Reproducibility

A reproducibility study was conducted following CLSI EP5-A2 at 3 sites incorporating a 7 member panel consisting of 5 serum pools (high negative, low positive and moderately positive) and 2 controls that were assayed for 5 days, 2 runs per day, 3 replicates per sample. Data from all 3 sites were combined to achieve SD and percent CV for repeatability (within-run), between run, between-day, between site and reproducibility (imprecision). Data are summarized in the following tables:

		Mean	Within-run			Between-run	
Sample	N	COI	SD ^{e)}	95 % CI ^{f)}	CV %	SD	CV %
HSP ^{g)} 06	90	1.02	0.01	(0.01, 0.01)	1.2	0.02	2.3
HSP 07	90	1.13	0.02	(0.02, 0.03)	1.9	0.03	2.6
HSP 08	90	0.94	0.01	(0.01, 0.02)	1.6	0.02	2.6
HSP 09	90	0.85	0.02	(0.01, 0.02)	1.9	0.02	2.5
HSP 10	90	3.24	0.09	(0.08, 0.11)	2.8	0.08	2.6
PreciControl Syphilis1	90	0.12	0.00	(0.00, 0.00)	2.4	0.00	2.4
PreciControl Syphilis2	90	4.76	0.08	(0.07, 0.10)	1.7	0.14	2.9

e) Standard deviation

f) Confidence Interval

g) HSP = human serum pool

		Mean	Between-day		Between-site		Reproducibility (Total)		
Sample	N	COI	SD	CV %	SD	CV %	SD	95 % CI	CV %
HSP 06	90	1.02	0.00	0.0	0.01	1.3	0.03	(0.02, 0.04)	2.9
HSP 07	90	1.13	0.00	0.00	0.01	0.8	0.04	(0.03, 0.05)	3.3
HSP 08	90	0.94	0.00	0.0	0.01	0.8	0.03	(0.02, 0.04)	3.1
HSP 09	90	0.85	0.00	0.0	0.01	1.2	0.03	(0.02, 0.04)	3.4
HSP 10	90	3.24	0.00	0.0	0.00	0.0	0.12	(0.11, 0.15)	3.8
PC ^{h)} Syphilis1	90	0.12	0.00	1.2	0.01	4.5	0.01	(0.00, 0.02)	5.7
PC Syphilis2	90	4.76	0.00	0.0	0.03	0.7	0.16	(0.13, 0.21)	3.4

h) PC = PreciControl

Note: SD of zero due to variance contributed by particular component was below stated significant figure.

Endogenous interference

Potential interference was evaluated with bilirubin, hemoglobin, Intralipid, biotin, rheumatoid factors, human serum albumin, human IgG, IgA and IgM. No interference was observed for the listed substances up to the levels indicated in the table below.

Interferent tested	No interference up to
Bilirubin	≤ 1129 μmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.310 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/mL or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
Human serum albumin	≤ 10 g/dL
Human IgG	≤ 3.2 g/dL
Human IgA	≤ 2.8 g/dL
Human IgM	≤ 1.0 g/dL

Specimens that contain biotin at a concentration of 1200 ng/mL, demonstrate less than or equal to 10 % negative bias in COI values. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day¹⁵ and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.¹⁶

Drug interference

A drug interference study was performed with 16 commonly used therapeutic drugs. No interference was observed at the levels tested.

Drug tested	No interference up to
Acetylcysteine	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

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Drug tested	No interference up to
Phenylbutazone	400 mg/L
Doxycycline	50 mg/L
Acetylsalicylic Acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L

Analytical specificity

Specimens from individuals diagnosed with other diseases were obtained from commercial vendors. The results of the study are shown below.

Potential Interference	Number tested	Number of reactive samples
Borrelia	50	0
EBV IgG	21	1
Rubella IgG	17	1
E. coli	18	1
Hepatitis A	25	1
Hepatitis B	10	2
Hepatitis C	13	0
ANA	15	1
RF	19	0
HSV-1/2	12	1
CMV IgG	20	0
HIV-1	25	1
Toxo IgG	21	0
Total	266	9

The 9 samples that were reactive with the Elecsys Syphilis assay were confirmed as coinfecting and positive for anti-*Treponema pallidum* antibodies with another syphilis total antibody assay. No cross-reactivity was found.

Clinical performance

A total of 2282 prospectively collected specimens from the intended use population were tested at 3 sites using the Elecsys Syphilis assay, including 1524 subjects sent for routine syphilis testing, 457 HIV positive subjects and 301 pregnant women. In addition, all samples were tested according to a composite testing algorithm using FDA-cleared tests that included the predicate syphilis immunoassays, the Rapid Plasma Reagin (RPR) non-treponemal specific assay and the *Treponema pallidum* Particle Agglutination *Treponema*-specific assay.

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

Predicate Syphilis Immunoassay	RPR	TPPA	Final Comparator Results	Elecsys Syphilis	N
NR ⁱ⁾	NR	N/A ^{j)}	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

Predicate Syphilis Immunoassay	RPR	TPPA	Final Comparator Results	Elecsys Syphilis	N
Reactive	NR	NR	Negative	Reactive	9
Reactive	NR	Reactive	Positive	Reactive	166
Reactive	Reactive	N/A ^{j)}	Positive	Reactive	62

i) NR = Non-reactive

j) test not performed

The comparison between the Elecsys Syphilis results and the final comparator results for the prospective specimens in the intended use population is shown in the following table.

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

The positive percent agreement was 100 % (228/228) with a 95 % confidence interval of 98.40 to 100 %. The negative percent agreement was 99.22 % (2038/2054) with a 95 % confidence interval of 98.74 to 99.58 %. The percent agreement for the individual cohorts is given below.

Percent agreement by category for prospective cohorts

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

Clinical performance in retrospective medically diagnosed individuals

Clinical performance in the pre-selected retrospective cohort included 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 given in the following table.

Performance of Elecsys Syphilis compared with Final Comparator

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

The positive percent agreement was 98.73 % (155/157) with a 95 % confidence interval of 95.47 to 99.85 %. The negative percent agreement was 100 % (12/12) with a 95 % confidence interval of 73.54 to 100 %. The percent agreement for the individual cohorts is given below.

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Percent agreement by category for retrospective cohorts

Cohort	Positive Percent Agreement		PPA 95 % CI	Negative Percent Agreement		NPA 95 % CI
	%	Ratio		%	Ratio	
Pregnant	100	15/15	78.20 to 100	N/A	0/0	N/A
Staged	98.6	140/142	95.00 to 99.83	100	12/12	73.54 to 100
Total	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 Elecsys Syphilis in subjects medically diagnosed with syphilis

Syphilis stage	Treatment status	N	Elecsys Syphilis Results	
			Reactive	Non-reactive
Primary	Treated	29	16	13 ^{k)}
	Untreated	25	25	0
Secondary	Treated	25	24	1
	Untreated	25	25	0
Latent	Treated	25	25	0
	Untreated	25	25	0

k) 12 of these samples also tested negative for syphilis with the composite testing algorithm

Clinical performance in pregnant women

A total of 316 pregnant female samples were tested in the study. Of these samples, 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.

Percent agreement in pregnant women

Cohort	Positive Percent Agreement		PPA 95 % CI	Negative Percent Agreement		NPA 95 % CI
	%	Ratio		%	Ratio	
Pregnant (Prospective)	N/A	0/0	N/A	100	301/301	98.78 to 100
1 st Trimester	N/A	0/0	N/A	100	100/100	96.38 to 100
2 nd Trimester	N/A	0/0	N/A	100	125/125	97.09 to 100
3 rd Trimester	N/A	0/0	N/A	100	76/76	95.26 to 100
Pregnant (Retrospective)	100	15/15	78.20 to 100	N/A	0/0	N/A
1 st Trimester	100	7/7	59.04 to 100	N/A	0/0	N/A
3 rd Trimester	100	8/8	63.06 to 100	N/A	0/0	N/A

In addition, 40 samples from pregnant women, non-reactive for *T. pallidum* antibodies, were spiked with serum with *T. pallidum* antibodies and analyzed with the Elecsys Syphilis assay. All samples tested reactive.

Performance in apparently healthy individuals

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

Results of Elecsys Syphilis assay in apparently healthy individuals

Gender	Elecsys Syphilis Results		
	Reactive	Non-reactive	Total
	N	N	
Female	9 (11.3 %)	71 (88.7 %)	80
Male	11 (8.53 %)	118 (91.5 %)	129
Total	20 (9.57 %)	189 (90.4 %)	209

Expected values

In this clinical study, there were a total of 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 *Treponema pallidum* antibodies in the study population. The distribution of the Elecsys Syphilis reactive and non-reactive results is summarized below by age and gender.

Age Range (years)	Gender	Elecsys Syphilis Results		
		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

Elecsys Syphilis

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For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
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SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
	Volume for reconstitution
GTIN	Global Trade Item Number

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