Elecsys Syphilis

cobas®

REF		Σ	SYSTEM
07251378 119	07251378500	300	**cobas e** 801

English

System information

Short name	A-CN (application code number)
SYPHILIS	10074

Intended use

Immunoassay for the in vitro qualitative determination of total antibodies to Treponema pallidum in human serum and plasma. The test is intended as an aid in the diagnosis of syphilis infection. This assay is also indicated as a donor screening test to detect antibodies to Treponema pallidum in serum and plasma specimens from individual human blood donors. This assay may also be used to detect antibodies to Treponema pallidum in serum and plasma specimens to screen individual organ donors when specimens are obtained while the donor's heart is still beating.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

Note:

Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit catalogue number : 07251378190 for the Syphilis. The last 3 digits -190 have been replaced by -119 for logistics purposes.

Summary

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

Syphilis is mainly transmitted sexually, but can also be transmitted from mother to fetus during pregnancy or birth. The global incidence of infection in 2008 was approximately 10.6 million and the total number of infections during that year was estimated to be 36.4 million.[2] In the USA the national infection rate rose to 6.3 cases per 100000 people, the highest rate since 1994.[3] Certain European countries have also seen increases in the rate of infection[4,5] and large localized outbreaks.[6] Each year, globally, an estimated 2 million pregnancies are affected.[7]

Congenital syphilis is still common in the developing world, as many women do not receive antenatal care or the scheme does not include syphilis screening.[8] Up to 80 % of syphilis infected pregnant women show adverse pregnancy outcomes.[7] The World Health Organization recommends all women to be tested at their first antenatal visit and again in the third trimester.[7] If they are positive, the recommendation also includes treatment of the partner.

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).[9]

The immune response to T. pallidum is the main driver of lesion development.[9] 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 detect antibodies against lecithin, cholesterol and cardiolipin, which are present in many syphilis patients.[1] Treponemal tests detect antibodies directed against T. pallidum antigens such as TpN47, TpN17 and TpN15, for IgM and IgG detection.[1] A positive treponemal antibody test result indicates exposure to T. pallidum but cannot distinguish between treated and untreated syphilis. Non-treponemal assays are useful to help distinguish between treated and untreated syphilis and are also used for monitoring the progression of disease and treatment response.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 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 II 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 ($Ru(bpy)_3^{2+}$)

Reagents – working solutions

The **cobas e** pack (M, R1, R2) is labeled as SYPHILIS.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 TP‑specific recombinant antigens (E. coli)~biotin, 1 bottle, 19.7 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)~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 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 1 (lyophilized), 1 bottle for 1.0 mL: Human serum, non-reactive for anti‑TP antibodies; preservative.

SYPHILIS Cal2 Positive calibrator 2 (lyophilized), 1 bottle for 1.0 mL: Human serum, reactive for anti‑TP antibodies; preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

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:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

All human material should be considered 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 applied were FDA-approved or cleared in compliance with the European Directive 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.[10, 11]

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

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

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.

Unless the entire volume is necessary for calibration on the analyzer, 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 2-8 °C or -20 °C for later use.

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

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack:	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	16 weeks

Stability of the calibrators:	
lyophilized calibrators	up to the stated expiration date
reconstituted calibrators at 2-8 °C	28 days
reconstituted calibrators at -20 °C	6 months (3 freeze/thaw cycles possible)
on the **cobas e** 801 analyzer 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, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma.

K_2-EDTA 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 (cutoff index) values from 0.0‑1.0 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 5 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 manufacturer.

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

Do not use heat‑inactivated samples.

Do not use samples and controls stabilized with azide.

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

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

The performance of the Elecsys Syphilis assay has not been established with cadaveric samples or body fluids other than serum and plasma.

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
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** 801 analyzer
- Distilled or deionized water

Accessories for the **cobas e** 801 analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 5694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the reconstituted calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

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 **cobas e** pack was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 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

Quality control

For quality control, use PreciControl Syphilis.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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 (signal sample/cutoff).

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Interpretation of the results

Numeric result	Result message	Interpretation/ further steps
COI < 1.00	Non-reactive	Negative for anti-TP antibodies, no further testing needed.
COI ≥ 1.00	Reactive	All initially reactive samples should be retested in duplicate with the Elecsys Syphilis assay.

Numeric result	Final result	Interpretation/ further steps
One or both of the duplicate retests have a COI ≥ 1.00	Repeatedly reactive	Must be confirmed according to recommended confirmatory algorithms.
Both of the duplicate retests have a COI < 1.00	Non-reactive	Negative for anti-TP antibodies.

Retesting of samples with an initial cutoff index ≥ 1.00 can be automatically performed (see section "**cobas e** flows").

cobas e flows
cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.
A **cobas e** flow is available to perform a repetition of measurements in duplicate automatically for samples with an initial cutoff index ≥ 1.00 (short name SYPH R).
Both sub-results and the overall result message will be reported.

Limitations – interference
The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.310 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 246 nmol/L or ≤ 60 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
IgG	≤ 32 g/L
IgA	≤ 2.8 g/dL
IgM	≤ 10 g/L
Human serum albumin	≤ 10 g/dL

Criterion: Mean recovery of positive samples within ± 15 %. Absolute deviation of samples with COI values from 0-1.0 within ± 0.2 COI.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

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

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

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.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

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.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera 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 801 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[c], negative	0.0836	0.00117	1.4	0.0013	1.4
HS, positive 1	0.932	0.0192	2.1	0.0243	2.3
HS, positive 2	1.11	0.0194	1.7	0.0246	2.2
HS, positive 3	3.55	0.0590	1.7	0.0692	2.0
HS, positive 4	46.4	0.908	2.0	0.982	2.1
PC[d] Syphilis 1	0.0891	0.00120	1.3	0.00139	1.4
PC Syphilis 2	4.80	0.0646	1.3	0.0973	2.0

c) HS = human serum

d) PC = PreciControl

Analytical specificity

236 samples containing antibodies against Borrelia, EBV, Rubella, HAV, HBV, HCV, HIV, CMV, HSV, E. coli, Toxoplasma gondii, ANA and rheumatoid factor, respectively, were tested with the Elecsys Syphilis assay. 227 samples were tested negative, 9 samples were tested positive for anti-syphilis antibodies (confirmed by Western Blot and other anti‑syphilis assays). No cross-reactivity was found.

Clinical sensitivity

A total of 924 samples from patients with suspected syphilis (diagnostic routine and blood screening) from Europe and Asia were tested with the Elecsys Syphilis assay. Four additional samples were excluded due to probable handling errors with banked samples. 922 samples were found to be positive for anti-syphilis antibodies (either clinically defined or confirmed by FTA‑Abs[e] and other anti‑syphilis assays). Two samples were found to be indeterminate. Overall, 922 samples were found to be repeatedly reactive (RR) with the Elecsys Syphilis assay. The 2 indeterminate samples were found to be non-reactive with the Elecsys Syphilis assay. The resulting sensitivity of confirmed positive samples is 100 %. The 95 % lower confidence limit was 99.60 %.

Cohort	N	Confirmed positive samples	Indeterm-inate samples	False negative samples[f]	Sensitivity[g] %
Primary syphilis	101	101	0	0	100
Secondary syphilis	124	124	0	0	100
Latent syphilis	470	470	0	0	100
Syphilis, stage unknown	229	227	2	0	100
Total[h]	**924**	**922**	**2**	**0**	**100**

f) Elecsys Syphilis assay (RR)

g) Sensitivity of confirmed positive samples

h) Four additional samples were excluded due to probable handling errors with banked samples.

e) FTA (Fluorescent Treponemal Antibody) - Abs (absorption)

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Clinical specificity

A total of 8079 samples (diagnostic routine and blood screening) from Europe and Asia were tested with the Elecsys Syphilis assay. 14 samples were found to be positive for anti-syphilis antibodies (confirmed by FTA‑Abs and other anti-syphilis assays), 8063 samples were found to be negative and 10 samples were found to be repeatedly false reactive with the Elecsys Syphilis assay (negative in FTA-Abs and other anti-syphilis assays). The resulting specificity in the study is 99.88 %. The 95 % lower confidence limit was 99.77 %.

Cohort	N	Confirmed positive samples	Confirmed negative samples	False negative samples[i)]	Specificity %
Diagnostic routine samples	3500	14	3486	7	99.80
Blood donor samples	4579	0	4577*	3	99.93
Overall specificity	**8079**	**14**	**8063***	**10**	**99.88**

i) Elecsys Syphilis assay (RR)

* Two samples were excluded due to indeterminate confirmation results.

References

1 Seña AC, White BL, Sparling PF. Novel Treponema pallidum serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010:51(6);700-708.

2 World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections – 2008, http://apps.who.int/iris/bitstream/10665/43782/1/9789241595858_eng.pdfpdf, 2012.

3 Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2014, http://www.cdc.gov/std/stats14/surv-2014-print.pdf.

4 European Centre for Disease Prevention and Control. Sexually transmitted infections in Europe 2013, http://ecdc.europa.eu/en/publications/Publications/sexual-transmitted-infections-europe-surveillance-report-2013.pdf, 2015.

5 Jebbari H, Simms I, Conti S, et al. Variations in the epidemiology of primary, secondary and early latent syphilis, England and Wales: 1999 to 2008. Sex Transm Infect 2011;87(3):191-198.

6 Righarts AA, Simms I, Wallace L, et al. Syphilis surveillance and epidemiology in the United Kingdom. Euro Surveill 2004;9(12):21-25.

7 World Health Organization. The global elimination of congenital syphilis: rationale and strategy for action, http://apps.who.int/iris/bitstream/10665/43782/9789241595858_eng.pdf, 2007.

8 Schmid G. Economic and programmatic aspects of congenital syphilis prevention. Bull World Health Organ 2004;82(6):402-409.

9 Lafond RE, Lukehart SA. Biological basis for syphilis. Clin Microbiol Rev 2006;19(1):29-49.

10 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

11 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
⟶	Volume after reconstitution or mixing
GTIN	Calibrator

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