Total antibodies to Treponema pallidum (T. pallidum, TP)

### English

**Intended use**

Immunoenzyme assay 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 electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

**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: 06923348190 for the Syphilis. The last 3 digits -119 have been replaced by -119 for logistics purposes.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 also can be transmitted from mother to fetus during pregnancy or birth. The global incidence of syphilis infection was 5.1 cases per 100000 men and 0.9 cases per 100000 women in 2005. In the USA, since 2005, syphilis has increased by 50 %, and some European countries have seen increases5 and large localized outbreaks.5 Each year, globally, an estimated 2 million pregnancies are affected.6

Congenital syphilis in the new born is still common in the developing world, as many women do not receive antenatal care or the scheme does not include syphilis screening.7 Up to 80 % of syphils infected pregnant women show adverse pregnancy outcomes resulting in an overall perinatal mortality rate of 40 %.7 Septicemia, abortion, or neonatal death can occur, and congenital syphilis is associated with significant morbidity. The World Health Organization recommends all women to be tested at their first antenatal visit and again in the third trimester.8 If they are positive, they recommend that their partners also be tested.9 Syphilis infection facilitates HIV infection.10

In the early stage of infection, the clinical diagnosis of syphilis can be very difficult.1 After the window period, blood tests using dark-field or fluorescence microscopy offer a more sensitive and easier alternative to visual inspection of the patient.1 Typically, the symptoms 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). A lengthy latent (asymptomatic) period follows. Eventually, tertiary syphilis ensues, characterized by the development of granulomatous dermal lesions, neurosyphilis, and/or cardiovascular syphilis (which can be fatal). The immune response to T. pallidum is the main driver of lesion development.11 The antibody response is directed not only against antigens specific to T. pallidum (treponemal antibodies) but also against antigens that are not specific to T. pallidum (non-treponemal antibodies), for example, antigens released during the cellular damage caused by the organism. Therefore, non-treponemal and treponemal tests are existing for the diagnosis of Syphilis.

Non-treponemal tests use antigens comprising lecthin, cholesterol, and purified cardiolipin to detect antibodies against cardiolipin, which are present in many syphils patients. Treponemal tests detect antibodies directed against T. pallidum proteins. A positive treponemal antibody test result indicates prior exposure to syphils. 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

### 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 complex8 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'-dipyridyl)ruthenium(II)-complex (Ru(bpy)3+)  

#### Reagents – working solutions

The reagent pack (M, R1, R2) is labeled as Syphilis. M Streptavidin-coated microtropcre (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/mL; MES buffer 50 mmol/L, pH 6.5; preservative. R2 TP-specific recombinant antigens (E. coli)-Ru(bpy)3 (black cap), 1 bottle, 9 mL: TP-specific recombinant antigens labeled with ruthenium complex 0.7 mg/mL; 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. 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 guideline.

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.

### Notes

1. European countries have seen increases and large localized outbreaks. Syphilis is mainly transmitted sexually, but also can be transmitted from mother to fetus during pregnancy or birth. The global incidence of syphilis infection was 5.1 cases per 100000 men and 0.9 cases per 100000 women in 2005. In the USA, since 2005, syphilis has increased by 50 %, and some European countries have seen increases and large localized outbreaks. Each year, globally, an estimated 2 million pregnancies are affected.

2. Syphilis infection facilitates HIV infection. In the early stage of infection, the clinical diagnosis of syphilis can be very difficult. After the window period, blood tests using dark-field or fluorescence microscopy offer a more sensitive and easier alternative to visual inspection of the patient. Typically, the symptoms 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). A lengthy latent (asymptomatic) period follows. Eventually, tertiary syphilis ensues, characterized by the development of granulomatous dermal lesions, neurosyphilis, and/or cardiovascular syphilis (which can be fatal). The immune response to T. pallidum is the main driver of lesion development. The antibody response is directed not only against antigens specific to T. pallidum (treponemal antibodies) but also against antigens that are not specific to T. pallidum (non-treponemal antibodies), for example, antigens released during the cellular damage caused by the organism. Therefore, non-treponemal and treponemal tests are existing for the diagnosis of Syphilis.

3. Non-treponemal tests use antigens comprising lecthin, cholesterol, and purified cardiolipin to detect antibodies against cardiolipin, which are present in many syphils patients. Treponemal tests detect antibodies directed against T. pallidum proteins. A positive treponemal antibody test result indicates prior exposure to syphils. 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.
Syphilis

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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.12,13

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

Reagent handling

The reagents in the kit are ready for use (except for Syphilis Ca1 and Syphilis Ca2) and are supplied in bottles compatible with the system. Syphilis Ca1 and Syphilis Ca2: 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.

Elecys 2010 and cobas e 411 analyzers: 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 calibration bottle set should be performed.

If necessary, freeze in aliquots; see section on MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers.

MODULAR ANALYTICS E170, 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 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: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for cobas 8000 systems only. If using a cobas 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument 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.

Stability of the reagent rackpack

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.

Stability of the reconstituted calibrators

either at -20 °C 6 months (3 freeze/thaw cycles possible)

or at 2-8 °C 28 days

on Elecsys 2010 and cobas e 411 at 20-25 °C up to 6 hours

on MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 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 in a sufficient number and found acceptable.

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

Li- heparin, Na- heparin, K2- EDTA, K3- EDTA, ACD, CPD, CP2D, CPDA and Na- citrate plasma as well as K2- EDTA plasma tubes containing separating gel.

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 14 days at 2-8 °C, 5 days at 25 °C, 12 months at -20 °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 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.

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 bottles labels

- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REFER 0692364190, PreciControl Syphilis, for 2 x 2 mL each of PreciControl Syphilis 1 and 2

- REFER 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles

- General laboratory equipment

- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer

- Distilled or deionized water

Accessories for Elecsys 2010 and cobas e 411 analyzers:

- REFER 11662988122, ProCell, 6 x 380 mL system buffer

- REFER 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution

- REFER 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive

- REFER 11933159001, Adapter for SysClean

- REFER 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels

- REFER 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:

- REFER 04880340190, ProCell M, 2 x 2 L system buffer

- REFER 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution

- REFER 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use

- REFER 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change

- REFER 03004899190, ProClean M, 5 x 600 mL detection cleaning solution

- REFER 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags

- REFER 03023150001, WasteLiner, waste bags

- REFER 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REFER 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
Syphilis
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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. MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreciClean 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 (MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers).

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 reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit 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
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 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.
Note: The controls are not barcode-labeled and therefore have to be run like external controls. All values and ranges have to be entered manually. Please refer to the section “QC” in the operator's manual or to the online help of the instrument software.
Non-barcode labeled controls: Only one target value and range for each control level can be entered in the analyzer. The reagent lot-specific target values have to be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run. The exact lot-specific target values and ranges are printed on the enclosed (or electronically available) value sheet in the reagent kit or PreciControl kit. Please make sure that the correct values are used.
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 syphilis-specific antibodies and do not need further testing.
Samples with a cutoff index ≥1.00 are considered reactive in the Elecsys assay.
All initially reactive samples should be redetermined in duplicate with the Elecsys Syphilis assay. If cutoff index values < 1.00 are found in both cases, the samples are considered negative for syphilis-specific antibodies. Initially reactive samples giving cutoff index values of ≥ 1.00 in either of the redeterminations are considered repeatedly reactive. Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms.

Limitations - interference
The assay is unaffected by icterus (bilirubin ≤ 1129 µmol/L or ≤ 66 mg/dL), hemolysis (Hb ≤ 0.310 mmol/L or ≤ 0.5 g/dL), lipemia (Intralipid ≤ 2000 mg/dL), human serum albumin ≤ 10 g/dL, human IgG ≤ 32 g/L, human IgM ≤ 10 g/L, human IgA ≤ 2.8 g/dL and biotin (≤ 246 nmol/L or ≤ 60 ng/mL).
Criterion: Mean recovery of positive samples within ± 15 %. Absolute deviation of samples with COI values from 0.00-1.0 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 interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
No false negative result due to high-dose hook effect was found with the Elecsys Syphilis assay.
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 titer 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 syphilis. 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 analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

<table>
<thead>
<tr>
<th>Elecsys 2010 and cobas e 411 analyzers</th>
<th>Mean COI</th>
<th>SD COI</th>
<th>CV %</th>
<th>SD COI</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS[1], negative</td>
<td>0.103</td>
<td>0.002</td>
<td>1.6</td>
<td>0.003</td>
<td>3.2</td>
</tr>
<tr>
<td>HS, positive 1</td>
<td>1.01</td>
<td>0.028</td>
<td>2.8</td>
<td>0.033</td>
<td>3.2</td>
</tr>
<tr>
<td>HS, positive 2</td>
<td>1.12</td>
<td>0.018</td>
<td>1.6</td>
<td>0.022</td>
<td>1.9</td>
</tr>
<tr>
<td>HS, positive 3</td>
<td>0.99</td>
<td>0.171</td>
<td>1.7</td>
<td>0.262</td>
<td>2.6</td>
</tr>
<tr>
<td>HS, positive 4</td>
<td>50.2</td>
<td>0.986</td>
<td>2.0</td>
<td>1.24</td>
<td>2.5</td>
</tr>
<tr>
<td>PreciControl Syphilis1</td>
<td>0.106</td>
<td>0.003</td>
<td>2.4</td>
<td>0.004</td>
<td>4.1</td>
</tr>
<tr>
<td>PreciControl Syphilis2</td>
<td>9.45</td>
<td>0.101</td>
<td>2.1</td>
<td>0.161</td>
<td>3.2</td>
</tr>
</tbody>
</table>

c) HS = human serum
Total antibodies to Treponema pallidum (T. pallidum, TP)

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 found to be negative, 9 samples were found to be 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 infection (diagnostic routine and blood screening) from Europe and Asia were tested with the Elecsys Syphilis assay. Four additional samples were found 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 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 two 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 %.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS, negative</td>
<td>Mean COI 0.062</td>
<td>SD COI 0.001</td>
</tr>
<tr>
<td>HS, positive 1</td>
<td>1.10</td>
<td>0.017</td>
</tr>
<tr>
<td>HS, positive 2</td>
<td>1.19</td>
<td>0.014</td>
</tr>
<tr>
<td>HS, positive 3</td>
<td>11.1</td>
<td>0.146</td>
</tr>
<tr>
<td>HS, positive 4</td>
<td>54.6</td>
<td>0.910</td>
</tr>
<tr>
<td>PreciControl Syphilis 1</td>
<td>0.064</td>
<td>0.001</td>
</tr>
<tr>
<td>PreciControl Syphilis 2</td>
<td>5.36</td>
<td>0.082</td>
</tr>
</tbody>
</table>

Overall specificity
For all samples (routine co-horts and blood donation) 8079 samples were tested. 14 samples were found to be positive for an unknown disease. The specificity was 99.80 %.

**References**
13. 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:
### Contents of kit
- Analyzers/Instruments on which reagents can be used

### Reagent
- Reagent

### Calibrator
- Calibrator

### Volume after reconstitution or mixing
- Global Trade Item Number

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