Instruction For Use

【Product Name】

Syphilis (eCLIA)

【Order Information】

REF NO.	Package Size
0320509807	50T
0320509811	2×50T
0320509808	100T
0320509812	2×100T

【Intended Use】

Immunoassay for the in vitro qualitative determination of total antibodies to Treponema pallidum in human serum and plasma. The Determination of Syphilis is used for auxiliary diagnosis of Treponema pallidum infection, for screening of blood donations and screening test to prevent transmission of Treponema pallidum.

【Summary】

Syphilis is caused by the intracellular Gram-negative Treponema bacterium Treponema pallidum (TP) subspecies syphilis[1]. Syphilis is primarily transmitted sexually, but can also be transmitted from mother to fetus during pregnancy or childbirth. The global incidence of infections in 2008 was approximately 10.6 million, and the total number of infections that year was estimated at 36.4 million[2]. In the United States, the national infection rate rose to 6.3 cases per 100,000 people, the highest rate since 1994[3]. Infection rates and large localized outbreaks have also increased in some European countries. Every year, an estimated 2 million pregnant women are affected globally[4].

When a person is infected with syphilis, two types of antibodies are produced in the body. One is specific anti-syphilis antibody, which is a diagnostic indicator after infection[5]. Although specific, it does not reflect the patient's infection status. Antibodies persist long-term even after a patient is cured[6]. At present, there are many detection methods for syphilis, such as ELISA, gold-labeled immunochromatography and Western blotting, etc., using Nichols strain TP or purified, genetically recombined TP antigen to determine specific antibodies in serum[7-9]. The specificity of the test can reach about 99%, but false positives cannot be absolutely ruled out. Another type of antibody is anti-lipid antibody, which is produced by the human body after TP infects the human body and decomposes and destroys human tissues, and releases the decomposition products into the blood. The product released from the breakdown is cardiolipid, and the resulting antibody is reagin[10].

A positive treponemal antibody test result indicates exposure to TP 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.

10 µL of sample, biotinylated TP-specific recombinant antigens and TP-specific recombinant antigens labeled with ruthenium complex react to form a sandwich complex. 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 system reagent. 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.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles 0.45mg/mL; 0.1M PBS, ProClin™300;	1×1.8 mL	2×1.8 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Biotinylated TP-specific recombinant antigens 0.8 mg/L; 50 mM PBS, ProClin™300;	1×3.5 mL	2×3.5 mL	1×7.0 mL	2×7.0 mL
Reagent A (RA)	TP-specific recombinant antigens labeled with ruthenium 0.8 mg/L; 50 mM PBS, ProClin™300;	1×3.5 mL	2×3.5 mL	1×7.0 mL	2×7.0 mL
Syphilis Calibrators	Negative calibrator, non reactive for anti-Treponema pallidum antibodies, 50 mM PBS, ProClin™300;	Cal 1: 1×1.0 mL			
	Positive calibrator, reactive for anti-Treponema pallidum antibodies, 50 mM PBS, ProClin™300;	Cal 2: 1×1.0 mL			
Syphilis Control materials (Optional)	Negative control, non reactive for anti-Treponema pallidum antibodies; 50 mM PBS; ProClin™300;	Level 1: 1×1.0 mL			
	Positive control, reactive for anti-Treponema pallidum antibodies; 50 mM PBS; ProClin™300.	Level 2: 1×1.0 mL			

Traceability: This method can be traced to manufacturer's selected measurement procedures.

See the control material value sheet for the target values and ranges of control

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

Additional materials for Automated ECL Immunoassay Analyzers Series eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzers Series eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, o REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzers Series eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, o REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2 ºC-8 ºC.

Do not freeze.

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

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened store at 2 ºC~8 ºC	18 months
Store at 2 ºC~8 ºC after opening	8 weeks
Store on the analyzers at 2 ºC~15 ºC	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened store at 2 ºC~8 ºC	18 months
Store at 2 ºC~8 ºC after reconstituted	8 weeks
Store on the analyzers at 18 ºC~28 ºC	8 hours

Store calibrators and control materials upright in order to prevent the calibrators and control materials from adhering to the cap of the vial.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers Series: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

Human serum and plasma added with Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, Na-citrate anti-coagulants are recommended.

Blood samples should be collected by standard operation of venous puncture. After complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not in suggestion.

Samples can be stored for 7 days at 18 ºC-28 °C, 14 days at 2 ºC-8 °C, 12 months at -20 °C ± 5 °C. The samples may be frozen 5 times.

Ensure the samples, calibrators and control materials are at 18 ºC-28 °C prior to measurement.

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

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Syphilis test according to the operational manual.

Put the Syphilis reagent pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 minutes before testing.

Recommended ambient temperature:10 ºC~30 °C; Relative humidity: ≤ 80%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same reagent lot was used on the analyzer beyond 4 weeks;

(3) Control materials findings outside the defined limits;

(4) The Buffer of different lots are used.

Quality Control procedure

Use Syphilis Control Materials for control materials.

In addition, other suitable Control Materials are also applicable.

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

the defined limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for Quality Control.
Please make sure that the correct values are used.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of Syphilis Cal 1 and Syphilis Cal 2.
The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI).

【Result Interpretation】

Initial Results		
Concentration	Instrument Result	Retest Procedure
< 1.0 COI	Non-reactive	Not retest required
≥ 1.0 COI	Reactive	Retest in duplicate

Duplicate Retest Results	
Instrument Result	Specimen Result
both results non-reactive	Specimens are considered non-reactive for Anti-TP
one or both reactive	Specimen are considered repeatedly reactive for Anti-TP

Repeatedly reactive samples are recommended for further investigating by supplemental methods.

【Limitations】

Interference

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

Endogenous substances

Compound	Concentration tested
Bilirubin	≤66 mg/dL
Hemoglobin	≤500 mg/dL
Triglyceride	≤2000 mg/dL
Biotin	≤60 ng/mL
Rheumatoid factors	≤1500 IU/mL
Human serum albumin	≤10 g/dL
HAMA	≤250 ng/mL

No false negative result due to high-dose hook effect was found with the Syphilis (eCLIA).

Pharmaceutical substances

In vitro tests were performed on commonly used phamaceuticals of 5.7 mg/dL apicillin sodium salt, 3.5 mg/dL doxycycline, 1.2 mg/dL azithromycin, 66.1 mg/dL cefoxitin, 5.0 mg/dL cyclosporine, 6.1 mg/dL ascorbic acid, 12.0 mg/dL metronidazole, 40.0 mg/dL phenylbutazone, 2.0 mg/dL levodopa, 1.7 mg/dL methyldopa, 20.6 mg/dL acetaminophen, 50.1 mg/dL ibuprofen, 4.0 mg/dL theophylline, 96.6 mg/dL ceftriaxone, 65.3 mg/dL aspirin, 6.0 mg/dL rifampicin, 4.59 µg/mL lamivudine, 5.74 µg/mL tenofovir, 6.31 µg/mL efavirenz. No interference with the assay was observed.
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.

【Analytical Performance】

Specific performance data

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

Precision

Precision was determined using samples in a protocol (EP5-A3) of the CLSI. The following results were obtained:

Sample	Mean (COI)	Repeatability (%CV)	Within-Laboratory Precision (%CV)	Reproducibility (%CV)
Human Sample, negative	0.506	1.60%	4.46%	3.72%
Human Sample, positive 1	1.481	1.39%	5.11%	3.83%
Human Sample, positive 2	6.042	1.27%	4.86%	2.92%

Homogeneity of calibrators and control materials

Within-bottle homogeneity: The coefficient of variation (CV) ≤ 8%.
Between-bottle homogeneity: The coefficient of variation (CV) ≤ 10%.

Measured values of control materials

The measured results of supporting assigned control materials of the kit should be within the control materials range.

Analytical specificity

Samples containing potentially interfering substances or derived form high-risk groups were tested with the assay. The specimens containing antibodies against HAV, HBV, HCV, HIV, HEV, HSV, HBsAg, CMV and Toxoplasma gondii, Rubella virus, Escherichia coli and EB virus, elevated titers of rheumatoid factor, elevated titers of HAMA were tested, no cross reaction was observed.

Diagnostic sensitivity

Samples from Syphilis positive patients with different stages of the infection disease and unselected blood donors, hospitalised patients. The resulting sensitivity of confirmed positive samples was 100%. The 95% lower confidence limit was 98.90%.

Diagnostic specificity

Samples from unselected blood donors and hospitalised patients. The resulting specificity was 99.96%. The 95% lower confidence limit was 99.86%.

【Precaution and Warning】

1. The kit is only used for in vitro diagnostics.
2. Exercise the normal precautions required for handling all laboratory reagents.
3. All reagents and samples including specimen, reagents and calibrators should avoid foaming before and during test.
4. Safety data sheet available for professional user on request.
5. This product contains animal-sourced materials and may have potential biological risk. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.
6. Any serious incident that would be 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.
7. The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1), May cause an allergic skin reaction. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and Dispose of contents/container to an approved waste disposal plant.

【Literature References】

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【Symbol Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limitation		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Manufacturer】

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