Clinical Evaluation of Fully Automated Elecsys® Syphilis Assay for the Detection of Antibodies of *Treponema pallidum*

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Objective: The resurgence of syphilis in recent years has become a serious threat to the public health worldwide, and the serological detection of specific antibodies against *Treponema pallidum* (TP) remains the most reliable method for laboratory diagnosis of syphilis. The performance of the Elecsys® Syphilis assay, a brand new electrochemiluminescence immunoassay (ECLIA), was assessed by large amounts of samples in this study. **Methods:** In comparison with InTec assay, the Elecsys® Syphilis assay was evaluated in 146 preselected samples from patients with syphilis, 1803 clinical routine samples, and 175 preselected samples from specific populations with reportedly increased rates of false-positive syphilis test results. Discrepancy samples must be investigated by Mikrogen Syphilis recomline assay. **Results:** There was an overall agreement of 99.58% between two assays

(Kappa = 0.975). The sensitivity and specificity of the Elecsys® Syphilis assay were 100.0% (95% CI, 96.8–100.0%) and 99.8% (95% CI, 99.5–100.0%), respectively. The Elecsys syphilis assay displays better sensitivity (100%), specificity (99.8%), PPV (98.7%), and NPV (100%) in 2124 samples enrolled, compared with the InTec assay. **Conclusion:** Considering the excellent ease of use and automation, high throughput, and its superior sensitivity, especially in primary syphilis, the Elecsys® Syphilis assay could represent an outstanding choice for screening of syphilis in high-volume laboratories. However, more attention was still needed, or the results must be confirmed by other treponemal immunoassays. The new Elecsys® Syphilis assay is applied to patients with malignant neoplasm or HIV infection. J. Clin. Lab. Anal. 30:1164–1168, 2016. © 2016 Wiley Periodicals, Inc.

Key words: immunoassay; sensitivity; specificity; syphilis

INTRODUCTION

Syphilis, known as a sexually transmitted disease (STD) caused by *Treponema pallidum* subspecies *pallidum* (TP), is a health problem of increasing incidence in recent years. Up to 1,000 patients before operation and/or transfusion blood products are compulsively screened for anti-TP (TP antibodies) every workday in our hospital. Usually, syphilis diagnosis relies upon a combination of tests, including treponemal immunoassays and non-treponemal tests. The toluidine red unheated serum test (TRUST), *T. pallidum* particle agglutination assay (TPPA) plus InTec enzyme immunoassay (EIA) are employed in our hospital. Generally, syphilis screening can begin with InTec ELISA assay, TRUST, or the combination of TPPA and TRUST according to clinical requests (Fig. 1). Initial reactive results of EIA assays are reviewed in duplicated using the same assays to minor

false results, and then TPPA and TRUST are advised to confirm the infection and monitor the disease activity. To avoid the biological or technological false results of the TRUST assay, extra Intec EIA assay or formerly LIS results are performed or searched. Hence, the InTec ELISA test assay for TP (InTec Products, Inc., Xiamen, China) is available in our lab, a two-step DAGS ELISA utilizing the recombinant treponemal antigens (TpN15, TpN17, TpN47) to qualitatively detect the IgG and IgM antibodies to *T. pallidum* in human serum or plasma, may be the bottleneck in

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shortening the turn-around time (TAT) for its batch test mode. With the heavier burden of the syphilis screening, there is a critical demand for simple, rapid, and high-throughput detection of syphilis. The Elecsys® Syphilis assay is an electrochemiluminescence immunoassay (ECLIA), and displays an excellent ability to automate testing on high-throughput instrumentations (1). We evaluated the performance of the Elecsys® Syphilis assay as a screening test for syphilis in West China Hospital with different populations, compared with the InTec assays (EIA) available.

MATERIALS AND METHODS

Samples

In total, 2,124 samples were enrolled from West China Hospital. A total of 146 preselected samples were classified to primary (32 cases), secondary (21 cases), tertiary (12 cases), and latent syphilis (81 cases) according to the clinical and laboratory criteria (2). The specificities of the diagnostic assays were evaluated using 1,803 clinical routine samples. Assay interference was assessed with additional 175 preselected potential cross-reactive samples, including 45 samples from patients with autoimmune disease—rheumatoid

arthritis (25 cases) and systemic lupus erythematosus (20 cases), 25 samples from pregnant women, 65 samples from patients with tumors, and 40 samples from patients with viral infections, consisted of HIV, HBV, HCV, and EBV. Total sample volumes should be sufficient to enable a repetition of experiments or further methods (in case discrepancies found).

Assays

Each sample was measured in parallel with Elecsys® syphilis assay and Intec assays. Elecsys® Syphilis assay (Roche Diagnostics, Mannheim, Germany), a one-step double-antigen sandwich (DAGS) qualitative ECLIA performed on fully automated analyzers Cobas e601 or MODULAR E170 analyzers (Roche Diagnostics), uses the recombinant treponemal TpN15, TpN17, and TpN47 antigens to simultaneously detect both the anti-treponemal total antibodies, including IgG and IgM antibodies.

Discrepancy Resolution and Confirmatory Testing

If individual results do show discrepancies with two of comparative methods, discrepancy samples must be investigated by Mikrogen Syphilis recomline assay

Fig. 1. The testing algorithms of syphilis in our hospital. LIS, laboratory information system; TRUST, The toluidine red unheated serum test (TRUST); TPPA, *T. pallidum* particle agglutination assay;

(Mikrogen Diagnostic, Martinsried, Germany), which determine specific IgG and IgM antibodies to individual *T. pallidum* antigens. Samples were tested by professionals according to the manufacturer's instructions and a test was considered positive when at least two of the six diagnostic bands corresponding to Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17, and Tp15 were clearly recognized.

Statistical Analysis

The intraassay variation in the Elecsys® Syphilis assay was evaluated by replicate measurements (*n* = 20) of patient samples at three levels (negative, weak positive, and strong positive) in 5 days (4 times a day). The Kappa coefficient was calculated as a measure of agreement between two assays. The statistical analysis of diagnostic indexes and representation was performed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

The precision of the Elecsys Syphilis was less than 3%, evaluated by replicate measurements of patient samples at three levels (negative, weak positive, and strong positive) in 5 days. The sensitivities of the Elecsys® Syphilis assay and InTec EIA assay for syphilis were 100%, assessed in 146 known TP antibodies-positive samples from patients in various clinical stages.

In total, 1,803 routine samples were tested for specificity of the Elecsys® Syphilis assay and InTec ELISA assay for TP. A total of 43 true-positive results were found, hence the prevalence of anti-TP-positive rate is 2.38% in our hospital. One false-positive result was found in Elecsys® Syphilis assay. Meanwhile, five false-positive results and one false-negative one were found in InTec assay. The overall specificity of the Elecsys® Syphilis assay in routine samples was 99.9%, a litter higher than that of InTec assay (99.7%). There were two Elecsys® Syphilis false-positive results from patients with gastric carcinoma or HIV infection. Hence, there are nine discrepancy specimens in total.

The only one false-negative results InTec may be deemed as borderline (*S/CO* = 0.886).

Among 2,124 serum samples tested for anti-TP, the Elecsys® Syphilis assay demonstrated an overall agreement of 99.6% (2,115/2,124; 95% CI 99.2–99.8) with InTec ELISA test kit for TP (*kappa* = 0.975). The sensitivity, specificity, and positive and negative predictive values of Elecsys and InTec assays were calculated (Table 1). The Elecsys syphilis assay displays better sensitivity (100%) and specificity (99.8%), compared with the InTec assay.

DISCUSSION

The prevalence of syphilis in China varies greatly among population subgroups, from 0.65% in urban citizens (3) to 10.9% in homosexual men or gay (4). The prevalence of anti-TP-positive rate is 2.38% in our hospital. Beyond the wide use of enzyme immunoassays (EIA),the Elecsys® Syphilis assay has been arousing the interest for its nice performance-easy operation, fastness, and individual tests, which can help to shorten TAT according to our syphilis protocol. Elecsys® Syphilis with good precision less than 3% brought specificity (99.9%) in routine samples and sensitivity (100%) in preselected syphilis-positive samples. The overall specificity of Elecsys® Syphilis is a quite higher than those of HISCL anti-TP assay and Lumipulse G TP-N (99.37%) (5). The Elecsys® Syphilis assay demonstrated specificities of 97.4–100.0% in different populations, comparable to that of InTec assay for TP (99.7–100.0%). The Elecsys® Syphilis assay is a nice screening test for pregnant women, and patients with autoimmune disorders as well as patients with routine syphilis diagnostic requests. All those populations had reportedly false-positive serological testing results (6–9). For samples from patients with viral infections and malignant neoplasm or tumors, there was a slightly lower specificity. Viral infections are common in syphilitic patients, especially HIV, as *T. pallidum* can produce open lesions, which may result in an increased risk of acquiring HIV. In addition, HIV can adversely affect the serologic response to syphilis and

TABLE 1. Two by Two Table with 2,124 Samples from Which the Sensitivity, Specificity, and Positive and Negative Predictive Values are Calculated

		True results ^a		Se	Sp	PPV	NPV
		+	—				
Elecsys	+	221	3	1.00 (0.983, 1)	0.998 (0.995, 0.999)	0.987 (0.961, 0.997)	1.0 (0.998, 1)
	—	0	1900				
InTec	+	220	5	0.996 (0.975, 0.999)	0.997 (0.993, 0.999)	0.978 (0.949, 0.993)	0.9995 (0.997, 1)
	—	1	1898				

^aEach true-positive or true-negative result was defined either by Mikrogen Syphilis recomline assay or by consistence between Intec and Elecsys assay. PPV, positive predictive value; NPV: negative predictive value.

may affect the detection of infection (9,10). In a previous study by Martin et al., the specificity of the Elecsys® Syphilis assay in patients with confirmed HIV infection was reported to be 100.0% (95% CI, 98.4–100.0) (1). Nevertheless, in this study, one false-positive result was truly detected in 10 HIV-positive patients using Elecsys® Syphilis assay. More study was needed to evaluate the Elecsys® Syphilis assay with enlarged amount of samples in this special population. Furthermore, no potential cross-reactivity was observed in other 30 samples positive for HBV, HCV, or EBV. In the clinical settings, false reactions may occur in patients with malignant neoplasm during immune assay (6), but not enough attention was paid to this issue in the previous study (1). To make up the shortfall, 65 preselected samples from patients with different kinds of neoplasm were particularly included in our evaluation. In the results, a single sample from patient with gastric carcinoma was confirmed to be false positive using the Elecsys® Syphilis assay. However, our results do not necessarily suggest that neoplasm was the definite reason for cross-reactivity in this particular sample. To be safe, more attention should be paid when the new syphilis assay is applied to patients with malignant neoplasm. For syphilis serology, sensitivities vary depending on the type of test and stage of infection, with lower sensitivities in primary syphilis and late syphilis (11). Both the Elecsys syphilis and InTec ELISA test kit for TP showed 100% sensitivity with samples at all stages of infection, including the primary and late syphilis.

In the clinical setting, we often face with the problem that some samples with borderline s/co ratio results are hard to make decision, which may also

result in waste of time and resources for duplicate detection. From the distribution of s/co in 1,803 routine clinical samples (Fig. 2), there is clear discrimination between positive and negative results based on their s/co ratio using the Elecsys® Syphilis assay. The Elecsys® Syphilis assay was efficient to provide evidence for clinical decision making, and could reduce the need for confirmatory testing.

In conclusion, the Elecsys syphilis assay displays better sensitivity (100%), specificity (99.8%), PPV (98.7%), and NPV (100%) in 2,124 samples enrolled, compared with the InTec assay. The Elecsys® Syphilis assay perfectly agreed with the InTec ELISA test kit and was also compared favorably with or better than other CLIAs reported in the literature (12–14). Besides, the Elecsys® Syphilis assay demonstrates more priority in the terms of ease of use, high sensitivity in early syphilis, and less sera. A limitation of the study is that, although the test performed well for adult patients with primary, secondary, tertiary, and latent syphilis, patients with congenital syphilis were not included. As with all other treponemal assays, the Elecsys® Syphilis assay cannot distinguish among recent, remote, and previously treated infections. Therefore, its interpretation must combine with clinical findings and results from other tests for optimal use.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

The Roche Diagnostics GmbH (Shanghai, China) provided the kits and reagents used during the study.

Fig. 2. Distribution of the COI values of samples determined with the Elecsys® Syphilis assay. The number of borderline samples ($0.7 < \text{COI} < 3.0$) is 12 of 1,803 routine samples

Ethic statements

The Ethics Committee of West China Hospital of Sichuan University approved this study.

Informed consent

The study was based on existing laboratory human specimen bank. These samples were analyzed anonymously. For researchers, all personal or private information was blind. Written or oral informed consents can be exempted according to rules of Ethics Committee of West China Hospital of Sichuan University.

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