The Diagnostic Value and Performance Evaluation of Five Serological Tests for the Detection of *Treponema pallidum*

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Background: Syphilis is caused by the bacterium *Treponema pallidum* (TP). The aim of this study was to establish a clinical approach for serodiagnosis of syphilis by evaluating the performance and diagnostic value of five serological tests for the detection of TP. **Methods:** Five tests were used to test the serum from syphilis patients and control patients, namely rapid plasma reagin (RPR) test, toluidine red unheated serum test (TRUST), TP passive particle agglutination assay (TPPA), TP-specific enzyme-linked immunosorbent assay (TP-ELISA), and TP-specific chemiluminescent immunoassay (TP-CMIA). **Results:** The sensitivity and diagnostic efficiency of TPPA (96.25%/98.38%), TP-ELISA (100%/95.41%), and TP-CMIA (100%/94.86%) were significantly higher than that of RPR (73.13%/86.22%) and TRUST (73.75%/86.49%) ($P < 0.05$). The minimum detectable concentrations for the five tests were 30 mIU/ml, 20 mIU/ml, 15 mIU/ml, 150 mIU/ml, and 150 mIU/ml,

respectively. According to receiver operating characteristic (ROC) curve, the optimal cut-off values for syphilis diagnosis by TP-CMIA and TP-ELISA were 2.2 and 2.0 S/CO (where S/CO = Sample/calibrator cut off), and the area under the ROC curve (AUC) were 0.998 for TP-CMIA and 0.999 for TP-ELISA. The titers/positive rates for RPR and TRUST dropped from 1:4 (100%) to 1:1 (23.3%) (both $P < 0.05$) after treatment. However, there were no significant differences when we compared the positive rate of syphilis patients before and after treatment by TPPA, TP-ELISA, and TP-CMIA. **Conclusions:** Treponemal tests, such as TPPA, TP-ELISA, and TP-CMIA, are recommended for clinical routine screening of syphilis. However, nontreponemal tests, for example, RPR and TRUST, perform better in therapy response assessment. Serological test should be tailored to respective facilities and clinical demands. J. Clin. Lab. Anal. 28:204–209, 2014. © 2014 Wiley Periodicals, Inc.

Key words: *Treponema pallidum*; antibody; detection; performance; diagnostic value

INTRODUCTION

Syphilis is a chronic, sexually transmitted disease caused by the bacterium *Treponema pallidum* (TP). These are often concluded as the characteristic of syphilis: diverse clinical manifestations, uncertain periods of symptoms, and long infection phase. Recently, the number of cases has been rising (1). Two types of antibodies generate during TP infection. One is nontreponemal antibodies (usually against cardiolipin antigen, lipoidal, or reagin) that can be detected by rapid plasma reagin (RPR), toluidine red unheated serum test (TRUST), unheated serum reagin (USR), or Venereal Disease Research Lab-

oratory (VDRL) tests, and the other is treponemal-specific antibodies. Various diagnostic tests have been used for detection of such antibodies, for example, TP passive particle agglutination assay (TPPA), TP-specific

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enzyme-linked immunosorbent assay (TP-ELISA) and TP-specific chemiluminescent immunoassay (TP-CMIA), TP hemagglutination assay (TPHA), and fluorescent treponemal antibody-absorbed test (FTA-ABS) (2).

In the past, manual tests such as TPPA were extensively used in the detection of treponemal-specific antibodies. However, due to the inherent complexity of the experimental procedure and various performance-affecting factors, RPR and TRUST have been more commonly used for serological screening of syphilis in many clinical laboratories. Recently, the advances in TP-CMIA and TP-ELISA have overcome many problems caused by manual operation through the application of automated instruments. These methods have now been used in early serological screening because of their superior detection capacity including quantitative batch detection.

The aim of this study was to assess the values of five tests (RPR, TRUST, TPPA, TP-ELISA, and TP-CMIA) in syphilis diagnosis and therapy response assessment, incorporated with receiver operating characteristic (ROC) curve analysis.

MATERIALS AND METHODS

Study Subjects

A total of 160 syphilis patients were recruited in the 1st Affiliated Hospital of Fujian Medical University between April 2011 and August 2011. Of these, 100 were male and 60 were female with age range 17–90 (average age of 55 ± 13). One hundred twenty cases had active infection (with syphilis signs or symptoms) and 40 cases had latent infection.

A total of 210 nonsyphilis patients were also recruited during the same period (110 males and 100 females) with average age of 47 ± 17 (range 11–86). They were asymptomatic and without high risk of syphilis, and at the same time at least two treponemal tests were confirmed negative. Among the nonsyphilis patients, 110 had undergone preoperational screening (injured, pregnancy, cancer, infection, etc.), while 100 were confirmed by general health check before the study. The serological profiles of another cohort of 30 syphilis patients (active syphilis before initiation of therapy) were dynamically compared at their initial hospital visit and after 3 months treatment. The diagnosis of syphilis complied with IUSTI: 2008 European Guidelines on the Management of Syphilis (3).

Sample Collection and Storage

Venous blood samples (3 ml) were collected from the recruited patients in the morning under fasting condition. Once coagulated, blood serum was separated by centrifugation (3,000 r/min, 10 min). TRUST, RPR, TPPA, and

TP-CMIA tests were performed on these samples within 24 h of venipuncture. Part of the sera were frozen at -20°C and subjected for TP-ELISA testing after thawing at a later stage.

Reagents and Instruments

The TRUST kit was purchased from Shanghai Rongsheng-biotech (Batch No. 20110701, Shanghai, China), RPR kit was from Shanghai KHB (Batch No. 20110407, Shanghai, China), TPPA kit was from FUJIREBIO INC. (Batch No. VN10507, Tokyo, Japan), TP-ELISA kit was from Xiamen Asintech. (Batch No. 2010120408, Xiamen, China), TP-CMIA was performed on the Abbott ARCHETICT I2000 immunoassay analyzer facilitated with the corresponding syphilis diagnostic reagents (Batch No. 02133LP20, Wiesbaden, Germany).

Tests for Syphilis Diagnosis and Monitoring

Of the five tests, TRUST and RPR were agglutination assays, TPPA was a gelatin particle agglutination assay, TP-ELISA implemented enzyme-linked immunoassay (ELISA result > 1.0 S/CO positive) (where S/CO = Sample/calibrator cut off), and TP-CMIA was a chemiluminescence microparticle immunoassay (CMIA result > 1.0 S/CO positive). Experimental procedures were carried out according to the manufacturers' instructions. In-house quality control was conducted on five tests using standard materials from Beijing Controls & Standards Biotechnology Co., Ltd. (Beijing, China).

Determination of Minimum Detectable Concentrations

The criteria for minimum detectable concentration was determined by serial dilution of the standard material (initial concentration = 200 mIU/ml, Batch No. 201105001, Beijing Controls & Standards Biotechnology Co., Ltd.) with the negative serum. Every diluted sample was detected for five times and the lowest concentration reaching a detectable rate of at least 80% (positive results for at least four times) was concluded as the minimum detectable concentration. Take the TRUST test for example, when the standard material was diluted to 150 mIU/ml and the positive rate was 80%, then the standard material continued to be diluted to 100 mIU/ml and the positive rate was less than 80%, and 150 mIU/ml was considered as the minimum detectable concentration.

Statistical Analysis

To elucidate the diagnostic performance of the five tests for syphilis diagnosis and monitoring, the

TABLE 1. The Characteristics Comparison of Five Syphilis Serological Tests

Tests	Sensitivity (%)	Specificity (%)	Diagnostic efficiency (%)	Negative predicted value (%)	Positive predicted value (%)	Negative Likelihood ratio	Positive Likelihood ratio
RPR	73.13	96.19	86.22	93.60	82.45	0.28	19.20
TRUST*	73.75	96.19	86.49	93.65	82.79	0.27	19.36
TPPA [#]	96.25	100.00	98.38	100.00	97.22	0.04	∞ ^a
TP-ELISA [#]	100.00	91.90	95.41	90.40	100.00	0.00	12.35
TP-CMIA [#]	100.00	90.95	94.86	89.39	100.00	0.00	11.05

The results were compared with RPR test.

[#] $P < 0.05$, * $P > 0.05$.

^aInfinity. Positive Likelihood ratio = Sensitivity/(1-Specificity).

sensitivity, specificity, diagnostic efficiency, negative and positive predicted value, and negative and positive likelihood ratio were calculated. Comparisons between groups were performed using the χ^2 test. Besides, to evaluate the sensitivity and specificity between TP-ELISA and TP-CMIA, the ROC curve was generated in SPSS 13.0 and area under the ROC curve (AUC) was calculated. Values of $P < 0.05$ were considered to be significant.

RESULTS

Diagnostic Performance

The characteristics of five syphilis serological tests were evaluated. TP-CMIA and TP-ELISA showed the highest sensitivity, whereas TPPA showed the highest specificity and diagnostic efficiency. A comparison of the main characteristics of each test is shown in Table 1.

Minimum Detectable Concentrations

The minimum detectable concentrations for various syphilis serological tests are shown in Figure 1. The minimum detectable concentrations for TP-CMIA, TP-

ELISA, TPPA, RPR, and TRUST were 15 mIU/ml, 20 mIU/ml, 30 mIU/ml, 150 mIU/ml, and 150 mIU/ml, respectively.

Therapy Response Assessment

The changes in serological profiles pre- and posttreatments in 30 syphilis patients are shown in Table 2. The S/CO value and positive rates in RPR and TRUST tests decreased dramatically after treatments, whereas no significant differences were found in TPPA, TP-ELISA, and TP-CMIA tests.

Diagnostic Cut-off Point

The evaluation of diagnostic efficiency of TP-CMIA and TP-ELISA tests using ROC curve is shown in Table 3 and Figure 2. The optimal cut-off point for syphilis detection by TP-CMIA and TP-ELISA were 2.2 and 2.0 S/CO, with high sensitivity and specificity at 96.88%/99.05% and 98.75%/99.05%, respectively. No statistically significant differences were observed in their AUC values (0.998 for TP-CMIA and 0.999 for TP-ELISA) ($P > 0.05$).

DISCUSSION

Serological tests are currently the main methods for diagnosis of syphilis; however, the evaluation of these tests has shown inconsistent results in the literature (3–7), especially whether the diagnosis approach works for China population is still to be evaluated. This study has investigated the diagnostic values of five tests (RPR, TRUST, TPPA, TP-ELISA, and TP-CMIA) using samples from 160 syphilis and 210 nonsyphilis patients.

According to our results, the sensitivity, diagnostic efficiency, and minimum detectable concentration of treponemal tests clearly outperformed nontreponemal tests. The sensitivity and diagnostic efficiency were higher in TPPA, TP-ELISA, and TP-CMIA than in RPR and TRUST. The minimum detectable concentrations were found in the order of TP-CMIA < TP-ELISA < TPPA < RPR ≤ TRUST. There was no major variation in specificity

Fig. 1. The minimum detectable concentrations for syphilis serological tests. The highest concentrations were found in RPR and TRUST (150 mIU/ml), followed by TPPA and TP-ELISA (30 mIU/ml and 20 mIU/ml) and the lowest concentration was found in TP-CMIA (15 mIU/ml).

TABLE 2. The Difference in Serological Profiles Three Months Before and After Treatment in 30 Syphilis Patients

Methods	RPR		TRUST		TPPA Positive rate	TP-ELISA		TP-CMIA (S/CO)	
	Positive rate	Mean titer	Positive rate	Mean titer		Positive rate	$\bar{x} \pm SD$ (S/CO)	Positive rate	$\bar{x} \pm SD$ (S/CO)
Before treatment	100%	1:4	100%	1:4	100%	100%	20.48 $\pm$ 6.42	100%	15.47 $\pm$ 5.86
After treatment	23.3%* (7/30)	1:1*	23.3%* (7/30)	1:1*	100%	100%	19.24 $\pm$ 6.01 [#]	100%	14.14 $\pm$ 5.11 [#]

The mean titer or $\bar{x} \pm SD$ is missing for TPPA test in the table. [#] $P < 0.05$, * $P > 0.05$.

(90% in average), except for TPPA (100%). Hence, higher diagnostic value was found in treponemal tests compared to nontreponemal tests for syphilis screening. After TP infection, the appearance of nontreponemal antibodies is usually later than treponemal-specific antibodies. Furthermore, these antibodies may even diminish in late-stage disease or posttreatment (8). Therefore, nontreponemal tests are no longer recommended for syphilis screening. Nonetheless, TP-ELISA and TP-CMIA appear to be suitable for syphilis screening with their superiority in sensitivity, simple operation, and batch detection. TP-CMIA is particularly suitable for extensive application because of its automated operation (9). On the other hand, TPPA can be used for confirmatory tests owing to its extraordinary specificity.

In this study, the characteristics of five tests before and after 3 months treatment for 30 syphilis patients were also dynamically evaluated. We found that the nontreponemal antibodies in RPR and TRUST showed a reduction trend, maintaining only seven positive cases after the treatment, along with a reduced level in titers to different extents. This finding is consistent with the previous reports (10, 11). However, the positive rates for TPPA, TP-ELISA, and TP-CMIA remained at 100% before and after treatment, with the S/CO values in the latter two methods showing no significant statistical difference ($P > 0.05$). Therefore, treatment outcome should be assessed based on the titer changes in RPR and TRUST test for diagnosed syphilis patients. Nontreponemal tests have advantage over treponemal tests in treatment efficacy observation.

TABLE 3. The Sensitivity and Specificity of TP-CMIA and TP-ELISA at Different Cut-off Values in Patients with Syphilis

Diagnostic cut-off point (S/CO)	TP-CMIA				TP-ELISA			
	Number of controls ^a	Number of cases ^b	Sensitivity (%)	Specificity (%)	Number of controls ^a	Number of cases ^b	Sensitivity (%)	Specificity (%)
0	191	0	100.00	0.00	193	0	100.00	0.00
1.0	3	0	100.00	90.95	4	0	100.00	91.90
1.1	0	0	100.00	92.38	3	0	100.00	93.81
1.2	1	1	100.00	92.38	1	0	100.00	95.24
1.3	0	0	99.38	92.86	1	0	100.00	95.71
1.4	1	0	99.38	92.86	1	0	100.00	96.19
1.5	1	0	99.38	93.33	0	0	100.00	96.67
1.6	4	2	99.38	93.81	1	0	100.00	96.67
1.7	2	0	98.13	95.71	2	0	100.00	97.14
1.8	0	0	98.13	96.67	1	2	100.00	98.10
1.9	3	2	98.13	96.67	1	0	98.75	98.57
2.0^c	0	0	96.88	98.10	1	1	98.75	99.05
2.1	2	0	96.88	98.10	0	2	98.13	99.52
2.2^d	1	4	96.88	99.05	0	1	96.88	99.52
2.3	0	1	94.38	99.52	0	3	96.25	99.52
2.4	1	2	93.75	99.52	0	2	94.38	99.52
2.5	0	13	92.50	100.00	1	4	93.13	99.52
≥ 3.0	0	135	84.38	100.00	0	145	90.63	100.00

^aControls consist of 210 nonsyphilis patients.

^bCases consist of 160 syphilis patients.

^cThe optimal cut-off values for syphilis diagnosis by TP-ELISA.

^dThe optimal cut-off values for syphilis diagnosis by TP-CMIA.

Fig. 2. The ROC curve of TP-CMIA and TP-ELISA tests against syphilis diagnosis. The optimal cut-off points for syphilis diagnosis using TP-CMIA and TP-ELISA according to ROC curve were 2.2 S/CO and 2.0 S/CO. The sensitivity and specificity were 96.88%/99.05% for TP-CMIA and 98.75%/99.05% for TP-ELISA. The area under the ROC curve was 0.998 and 0.999, respectively, with no statistical differences found between the two tests, ($P > 0.05$).

In regard to the substantial advantages of operation simplicity, quantitative and short turnaround time of TP-ELISA and TP-CMIA, we conducted ROC analysis and revealed their high diagnostic values for syphilis. No statistical difference was found when comparing TP-CMIA with TP-ELISA in terms of optimal cut-off points (2.2 S/CO vs. 2.0 S/CO), sensitivity and specificity (96.88%/99.05% vs. 98.75%/99.05%), and area under the curve (0.998 vs. 0.999). The specificity for both tests reached 100% when 2.5 S/CO and 3.0 S/CO were

used for the diagnostic cut-off points. By taking into account the literature (5, 7), we here conclude and introduce our approach to syphilis serological tests (Fig. 3). It is worthy to mention that due to the differences in method selection and the definition of the diagnostic cut-off point, every laboratory should tailor their syphilis serological tests according to respective conditions and demands.

In general, we assessed the value of five tests for diagnostic and monitoring of syphilis and drew a flow chart for proper use of such serological tests in clinical settings in China. At the same time, the approach needs to be implemented for more clinical verification. We will increase the sample size in the follow-up study to verify the clinical values of the approach.

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CONFLICT OF INTEREST

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Fig. 3. The approach for serodiagnosis of syphilis. Treponemal tests (TP-CMIA or TP-ELISA) are recommended for initial clinical screening of syphilis. On one hand, if the initial screening result is much higher than 1.0 S/CO, the TP infection can be confirmed and on the other hand, if the initial screening result is slightly higher than 1.0 S/CO, TPPA is used as the confirmatory test. When a patient is diagnosed with syphilis, nontreponemal tests (RPR or TRUST) are suggested to assess treatment efficacy with clinical symptoms.

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