

# Performance Evaluation of CLIA for *Treponema Pallidum* Specific Antibodies Detection in Comparison with ELISA

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**Objectives:** In this study we aimed to evaluate the performance effects of chemiluminescence assay (CLIA) for *Treponema pallidum* specific antibodies detection, and to compare *T. pallidum* specific antibodies detection accuracy between CLIA and ELISA with TPPA (*T. pallidum* particle agglutination assay) as a confirmatory test. **Methods:** A total of 865 samples from suspected syphilis patients and preoperative patients were included, in which *T. pallidum* specific antibodies were simultaneously detected by CLIA and ELISA. Among them, 457 samples were determined by TPPA. **Results:** All coefficients of variation (CVs) of ELISA in high-, median-, and low-level samples were more than 5% and the maximum CV was 54.39% in the low-level sample. CVs of CLIA in different-level samples were all below 5%. Among the three assays the Spearman correlation and Kappa coefficients were 0.771 ( $P \leq 0.001$ ) and 0.854 ( $P \leq 0.001$ , CLIA vs.

ELISA), 0.806 ( $P \leq 0.001$ ) and 0.897 ( $P \leq 0.001$ , ELISA vs. TPPA), 0.937 ( $P \leq 0.001$ ) and 0.967 ( $P \leq 0.001$ , CLIA vs. TPPA), respectively. The area under the receiver operating characteristic curve (AUC) of CLIA was higher than that of ELISA (0.994 vs. 0.989) with TPPA as the confirmatory test. In 18 discrepant samples the consistency rate between CLIA and TPPA was elevated compared with that between ELISA and TPPA (72.22% vs. 27.78%,  $P = 0.008$ ). In gray zone, the consistency rate of CLIA with TPPA was higher than that of ELISA with TPPA (90.91% vs. 41.67%,  $P = 0.027$ ). **Conclusions:** Compared with ELISA, CLIA is more reliable, sensitive and accurate to detect serum *T. pallidum* specific antibodies. In the future it may be an alternative test with higher sensitivity to ELISA. J. Clin. Lab. Anal. 30:216–222, 2016. © 2015 Wiley Periodicals, Inc.

**Key words:** chemiluminescence assay (CLIA); *Treponema pallidum* particle agglutination assay (TPPA); enzyme immunoassay (EIA); *Treponema pallidum* specific antibodies; syphilis

## INTRODUCTION

*Treponema pallidum* is the pathogenic factor for syphilis, which is a sexually transmitted disease (STD) and has the similar clinical signs and symptoms to other infectious diseases. Since the diagnosis of syphilis is difficult due to its diverse clinical manifestations, the detection of etiology or serology is very important and helpful in syphilis diagnosis. Serological tests play vital roles in the accurate diagnosis of syphilis and are divided into nontreponemal and treponemal tests. Treponemal tests are directed against *T. pallidum* proteins with high specificity; these include fluorescent treponemal antibody-absorption (FTA-ABS), *T. pallidum* hemagglutination assay (TPHA), *T. pallidum* particle agglutination assay (TPPA), and enzyme immunoassay (EIA) (1, 2). Among them FTA-ABS and TPPA are considered as confirmatory tests, but recently

some investigation in *Morbidity and Mortality Weekly Report (MMWR)* (3) mentioned that CDC of the United States recommended the FTA-ABS test not to be used to confirm discordant treponemal screening results because of some shortcomings such as lower specificity and probably lower sensitivity (4). The TPPA test is considered to be the most suitable confirmatory treponemal test according to previous published sensitivity and

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TABLE 1. Performance Characteristics of Three Different Assays

	LUMIPULSE G system	ELISA system	Agglutination assay
Principles of assays	Chemiluminescent immunoassay, CLIA (two steps)	Enzyme-linked immunosorbent assay, ELISA (two steps)	Gelatin particle agglutination assay (one step)
Analyzer	LUMIPULSE G1200 analyzer	Tecan freedom EVOlyzer automated ELISA system	No special analyzer
Reagents	Lumipulse® G TP-N reagent kits	ELISA Diagnostic Kits for antibody to <i>T. pallidum</i>	SERODIA®-TP•PA reagents
Test time	25 min	120 min	120 min
Antibodies' types	IgG and IgM	IgG and IgM	IgG and IgM
Coated antigen(s)	Recombination Tp15–17 and TpN47	<i>T. pallidum</i> antigens from genetic engineering	<i>T. pallidum</i> (Nichols Strain) antigen
Enzymes and substrates	ALP and AMPPD	HRP and TMB	/

ALP: alkaline phosphatase; AMPPD: 3-[2-spiroadamantane]-4-methoxy-4-[3-phosphoryloxy]-phenyl-1,2-dioxetane; HRP: horse radish peroxidase; TMB: tetramethylbenzidine

specificity data (5). However, the above-mentioned traditional treponemal tests have some inevitable limitations, such as complicated operation procedures, subjective results, time-consuming nature, and difficult automation. Therefore, development of a new analytic method with efficient testing, easy automation, high sensitivity and high specificity is critical for effective diagnosis of syphilis.

With the development of methodology, chemiluminescence assay (CLIA) has come into being. CLIA can automatically and quickly detect serum *T. pallidum* specific antibodies with high sensitivity and specificity for syphilis diagnosis (6–8). Previous studies mainly evaluated the diagnostic efficiency of EIA in *T. pallidum* specific antibodies detection (9,10). Till now most studies on methodology comparison focused on EIA with TPPA (5, 11) or CLIA with TPPA (12), and the researches on the consistency of performance effects for *T. pallidum* detection by ELISA and CLIA were scarce. Therefore, we aimed to evaluate the performance effects of CLIA for *T. pallidum* specific antibodies detection and to compare the detection accuracy between CLIA and ELISA with TPPA as a confirmatory test.

## MATERIALS AND METHODS

### Subjects

A total of 865 samples of suspected syphilis patients and preoperative patients collected from September 2012 to February 2013 in West China Hospital of Sichuan University were included; among the patients, there were 377 females and 488 males and their mean age was 47.48 years. All the samples were detected for *T. pallidum* specific antibodies by CLIA and ELISA, and 457 samples among them were detected by TPPA as the confirmatory test. Our study using human blood samples was performed

in accordance with the current revision of the Helsinki Declaration.

### Serum *T. pallidum* Specific Antibodies Determination

Serum was separated within 3 h after drawing and stored for 1 week at  $-20^{\circ}\text{C}$ . LUMIPULSE G1200 analyzer and Lumipulse® G TP-N reagent kits (Fujirebio Diagnostics, Tokyo, Japan) as well as Tecan freedom EVOlyzer automated ELISA system (Tecan Group, Männedorf, Switzerland) and ELISA Diagnostic Kits for Antibody to *T. pallidum* (InTec Products, Xiamen, China) and SERODIA®-TP•PA reagents (Fujirebio Diagnostics, Inc. Tokyo, Japan) were used. All assays were carried out according to the manufacturers' standard procedures. Detailed performance characteristics of three different assays were described in Table 1. According to the manufacturers' suggestions and our clinical practical experiences, the value of S/C.O. (the optical density ratio of sample to cut-off) between 0.8 and 1.2 and C.O.I (cut-off index) value between 0.9 and 1.1 were considered gray zones of ELISA and CLIA, respectively.

CLIA automatically detected serum *T. pallidum* specific antibodies on LUMIPULSE G1200 system. A total of 50  $\mu\text{l}$  serum or TP-N calibrator reacted with 250  $\mu\text{l}$  microparticle coated with Tp15–17 and TpN47 antigens, then with 250  $\mu\text{l}$  ALP-conjugated Tp15–17 and TpN47 antigens formed antigen-antibody-antigen complex. With the substrate AMPPD and under 477 nm, the complex was detected by chemiluminescence method.

ELISA detected serum *T. pallidum* specific antibodies on Tecan freedom EVOlyzer automated ELISA system with InTec ELISA kits. A total of 100  $\mu\text{l}$  sample, positive control, and negative control reacted with genetic engineering antigens coated microplate, and then reacted

with HRP-labeled genetic engineering antigens. With the substrate TMB and under 450 nm, the optical density was automatically detected.

TPPA was based on the agglutination of colored gelatin particle carriers sensitized with *T. pallidum* (Nichols Strain) antigen. Serum samples were serially diluted in sample diluent in microplate wells. Sensitized gelatin particles were added to respective wells and the contents of the plate were mixed by hand. The mixture was incubated for 2 h at room temperature. Serum containing specific antibodies formed a smooth mat of agglutinated particles in the microtitration tray. A compact button formed by the settling of the nonagglutinated particles characterized negative reactions.

## Statistics

All data were analyzed by statistical software SPSS 13.0. Kappa test was conducted to evaluate the consistency of qualitative results. Spearman correlation was used to show the correlation of quantitative results. With TPPA as the confirmatory test for *T. pallidum* specific antibodies detection, receiver operating characteristic (ROC) curve analysis was made to evaluate the areas under the ROC curve (AUCs), sensitivity and specificity of ELISA and CLIA in *T. pallidum* specific antibodies detection. Chi-square test or Fisher's exact test was performed to analyze the qualitative data or categorical variable comparison. *P*-value <0.05 was considered statistically significant.

## RESULTS

### Imprecision Analysis of ELISA and CLIA for Detecting *T. pallidum* Specific Antibodies

High-, median-, and low-concentration samples were selected to be continuously detected "20 times" by ELISA and CLIA. The detection showed that all coefficients of variation (CVs) of ELISA in high-, median-, and low-level samples were more than 5% and the maximum CV was 54.39% in the low-level sample. CVs of CLIA in different-level samples were all below 5% and less than CVs of ELISA in all the samples (Table 2).

### Consistency Analysis of CLIA and ELISA in Quantitative and Qualitative Results

In quantitative data or numerical variable, consistency analysis showed that Spearman correlation coefficient between CLIA and ELISA was 0.771 ( $P \leq 0.001$ , Fig. 1a) and when data were described as qualitative or categorical variable Kappa coefficient was 0.854 ( $P \leq 0.001$ , Table 3).

Under the manufacturers' cutoff in 865 samples, there were 30 discrepant results between CLIA and ELISA. And excluding the data in gray zone, in 833 samples 18 sera results were discrepant between CLIA and ELISA (Table 3). TPPA analysis showed that in 18 discrepant samples the consistency rate between CLIA and TPPA was significantly higher than that between ELISA and TPPA (72.22% (13/18) vs. 27.78% (5/18),  $P = 0.008$ ). Further analysis showed that in 18 discrepant samples the true-positive consistency rate of CLIA (12/14, 85.71%) was higher than that of ELISA (2/14, 14.29%;  $P \leq 0.001$ ), and the true-negative consistency rate of CLIA (1/4, 25%) was lower than that of ELISA (3/4, 75%;  $P \leq 0.001$ ) compared with TPPA.

### ROC Analysis of CLIA and ELISA with TPPA as the Confirmatory Test

AUC, specificity and sensitivity of CLIA and ELISA were calculated with TPPA as the confirmatory test. It showed that AUC of CLIA (0.994,  $P \leq 0.001$ ) was higher than that of ELISA (0.989,  $P \leq 0.001$ ; Fig. 1d). Under the manufactures' cutoff, the sensitivity of CLIA was higher than that of ELISA (94.07% vs. 82.96%) and the specificity of CLIA was similar to that of ELISA (99.07% vs. 99.38%). In quantitative data, Spearman correlation coefficient between CLIA and TPPA ( $r_s = 0.937$ ,  $P \leq 0.001$ ) was higher than that between ELISA and TPPA ( $r_s = 0.806$ ,  $P \leq 0.001$ ; Fig. 1b and 1c).

When excluding the gray-zone data, the sensitivity and specificity of CLIA were 91.11% and 98.45%, and those of ELISA were 87.40% and 99.37%. The consistency analysis of qualitative data showed Kappa coefficient between CLIA and TPPA was higher than that between ELISA and TPPA (0.967 ( $P \leq 0.001$ ) vs. 0.897 ( $P \leq 0.001$ ), Table 4). The false-positive rates of CLIA and ELISA were similar (0.94% (3/320) vs. 0.63% (2/318)), and the false-negative rate of CLIA was lower than that of ELISA (2.38% (3/126) vs. 12.60% (16/127),  $P = 0.002$ ).

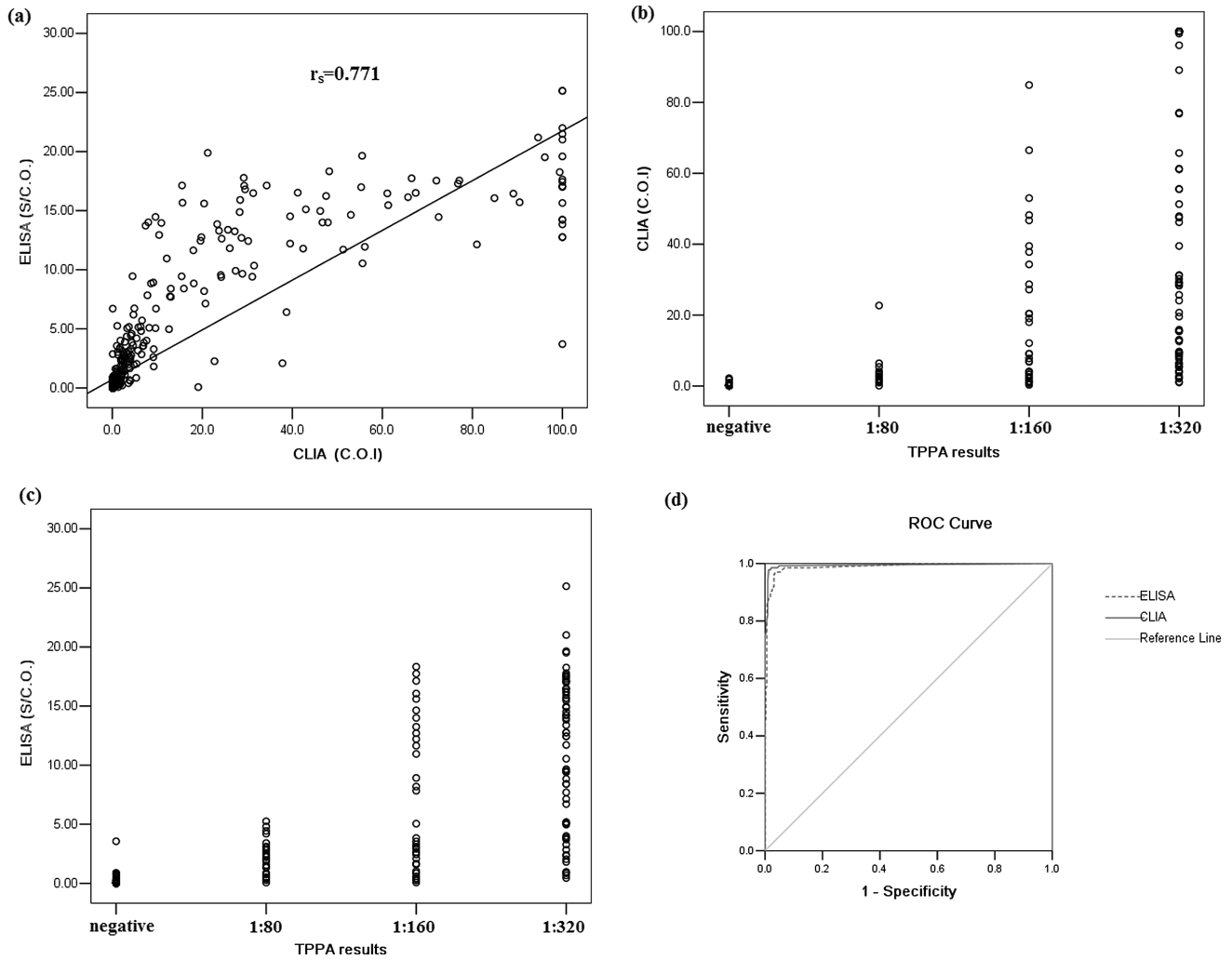
**TABLE 2. Imprecision Analysis of ELISA and CLIA for Detecting *T. pallidum* Specific Antibodies**

		Low concentration <sup>a</sup>	Median concentration <sup>b</sup>	High concentration <sup>c</sup>
CV (%)	ELISA	54.39	10.63	6.79
	CLIA	4.42	1.43	2.09

<sup>a</sup>Mean low concentration: CLIA—C.O.I = 0.1, ELISA—S/C.O. = 0.004.

<sup>b</sup>Mean median concentration: CLIA—C.O.I = 11.7, ELISA—S/C.O. = 7.44.

<sup>c</sup>Mean high concentration: CLIA—C.O.I = 73.76, ELISA—S/C.O. = 17.01.



**Fig. 1.** Quantitative analysis of serum *T. pallidum* specific antibodies detected by ELISA and CLIA . (a) Correlation of serum *T. pallidum* specific antibodies' levels between ELISA and CLIA. (b and c) Correlation of serum *T. pallidum* specific antibodies' levels between CLIA or ELISA and TPPA. (d) ROC analysis of CLIA and ELISA for serum *T. pallidum* specific antibodies detection with TPPA as the confirmatory test.

**Accuracy Analysis in Gray-Zone Data of ELISA and CLIA**

It was hard to define gray-zone data as true positive or true negative. Here TPPA was as the confirmatory test to detect serum *T. pallidum* specific antibodies. Compared with TPPA, the consistency rate of CLIA in gray-zone data was significantly higher than that of ELISA (90.91% (10/11) vs. 41.67% (5/12),  $P = 0.027$ ); true-positive rate in gray zone of CLIA also was strikingly higher than that of ELISA (81.82% (9/11) vs. 8.33% (1/12),  $P = 0.001$ ) and true-negative rate of CLIA was lower than that of ELISA (9.09% (1/11) vs. 33.33% (4/12),  $P = 0.317$ ; Table 4). Further analysis showed that 54.50% data in gray zone of CLIA were in TPPA low titer level (1:80), and 50% data in gray zone of ELISA were in TPPA median (1:160) and high titer levels (1:320, Table 5).

**TABLE 3. Kappa Test Analysis Between CLIA and ELISA**

		CLIA		
		Negative	Gray zone	Positive
ELISA	Negative	649	6	15
	Gray zone	10	4	7
	Positive	3	5	166
Kappa coefficient		0.854 ( $P \leq 0.001$ )		

**DISCUSSION**

Serological tests, especially treponemal tests, play an important role in syphilis diagnosis. Reverse sequence screening test for *T. pallidum* specific antibodies, in which sera are tested first by a treponemal EIA, is widely used

**TABLE 4. Comparison of ELISA and CLIA with TPPA Using Serum Specimens**

Assays and results	TPPA results (N)		%Sensitivity (95% CI)	%Specificity (95% CI)	%agreement (95% CI)	Kappa values (P-value)
	Negative	Positive				
CLIA			91.11 (88.33–93.54)	98.45 (97.12–99.38)	96.28 (94.35–97.82)	0.967 ( $P \leq 0.001$ )
Negative	317	3				
Positive	3	123				
Equivocal	2	9				
Low value <sup>a</sup>	1	0				
High value <sup>b</sup>	1	9				
ELISA			87.40 (84.20–90.28)	99.37 (98.44–99.89)	93.44 (90.99–95.52)	0.897 ( $P \leq 0.001$ )
Negative	316	16				
Positive	2	111				
Equivocal	4	8				
Low value <sup>a</sup>	4	7				
High value <sup>b</sup>	0	1				

<sup>a</sup>Value was lower than the manufacturer's cutoff of CLIA or ELISA.

<sup>b</sup>Value was higher than the manufacturer's cutoff of CLIA or ELISA.

**TABLE 5. ELISA and CLIA Gray Zone Data Analysis Compared with TPPA**

	TPPA			
	Negative	Positive		
		1:80	1:160	1:320
ELISA gray zone	4(33.30%)	2(16.70%)	3(25.00%)	3(25.00%)
CLIA gray zone	2(18.20%)	6(54.50%)	2(18.20%)	1(9.10%)

for syphilis serodiagnosis to reduce the time and labor required for syphilis screening. And EIA, especially ELISA and CLIA, will gradually become the critical screening treponemal test. ELISA, an important assay of EIAs, is considered as a traditional assay to detect serum *T. pallidum* specific antibodies, and especially based on its high sensitivity and specificity, it has been widely used since 1980s (13). CLIA, a newly developed method, can allow for rapid detection of *T. pallidum* specific antibodies on random-access analyzers with higher precision, sensitivity and specificity (6, 7). And TPPA, as the confirmatory test, can ensure its sensitivity and specificity for diagnosing syphilis infection by using *T. pallidum* (Nichols Strain) as antigen. Table 1 presented the characteristics of ELISA, TPPA, and CLIA, which indicated that CLIA was less time consuming and easy to handle compared with ELISA and TPPA. It also indicated that different assays applied different antigens and analysis systems to detect serum *T. pallidum* specific antibodies. CLIA and ELISA just used some *T. pallidum* antigen peptides, while in TPPA coated-antigen was the Nichols strain *T. pallidum*. As it

was known that the difference of coated antigen could influence sensitivity and specificity of assays. Here we expected to make the performance evaluation of CLIA in detecting *T. pallidum* specific antibodies compared with ELISA and TPPA.

Because treponemal tests were used to diagnose syphilis, the test stability was important. Table 2 demonstrated that CLIA was a more stable assay in detecting different *T. pallidum* specific antibodies levels (all CVs (%) < 5%), and ELISA results were diverse, especially in low-level detection (which was close to background level and easily influenced by many factors), and CVs of ELISA in median or high level were still higher than 5%.

Some reports showed that in the population with low prevalence of syphilis there was higher discordant percentage and false-positive percentage among different tests (3). So in our subjects the suspected syphilis patients and preoperative patients were included to avoid the different prevalence of syphilis in different population.

CLIA and ELISA were both EIAs, and ELISA was the traditional test. Here we made a consistency analysis between ELISA and CLIA in a prospective study with 865 sera. Because some data of CLIA were more than its upper limit, scatter figure was limited to show the correlation between CLIA and ELISA in abnormal distributed data, and Spearman correlation analysis would be a more proper method to evaluate their correlation. Here Kappa test (Kappa = 0.854) and Spearman correlation analysis ( $r_s = 0.771$ ) both indicated that there was a good consistency between CLIA and ELISA.

Further, we used TPPA as the confirmatory test to assess CLIA and ELISA performance characteristics. In



quantitative and qualitative analysis, CLIA showed higher consistency with TPPA compared with ELISA; especially as a serodiagnosis test CLIA had low false-negative rate (2.38%) and false-positive rate (0.94%) to guarantee the lower misdiagnosis and missed diagnosis rates. Figure 1d indicated that with TPPA as the confirmatory test both CLIA and ELISA had good diagnosis efficiency (all the AUCs  $\geq 0.95$ ), and CLIA was better. Compared with other studies (5, 6, 14–17), the sensitivity and specificity of CLIA and ELISA in our study were close or similar to others<sup>7</sup>—which indicated that the difference of reference/confirmatory tests may not be the critical factor to influence the sensitivity and specificity of evaluated assays. Wellinghausen N et al. and we both appraised diagnostic efficiency of CLIA with different confirmatory tests (FTA-ABS in Wellinghausen N et al.'s study and TPPA in our study). We have drawn a common conclusion that CLIA was a good screening test for syphilis diagnosis with good sensitivity and specificity (more than 90%). Here we speculated that the differences of coated antigens and analyzers or detection systems between ELISA and CLIA could be important factors to influence the sensitivity of ELISA. Some study demonstrated that CLIA might have a role either as a diagnostic screening test or as a confirmatory test following a nontreponemal screening test (6). However, we thought CLIA may be more appropriate for a screening test, not a perfect confirmatory test for syphilis diagnosis due to the coated nonintact *T. pallidum* antigen in CLIA. The nonintact *T. pallidum* antigen theoretically could cause false-positive results and influence the specificity and sensitivity of CLIA, which is shown in Table 4.

Gray-zone definition and interpretation were always problems for diagnostic test. Here Table 4 showed that CLIA had a higher accuracy and sensitivity with TPPA in all data analysis or excluding gray-zone data analysis than ELISA. And discrepant results' analysis demonstrated that CLIA had higher diagnostic accuracy rate (72.22%) and positive consistency rate (85.71%) with TPPA than ELISA. Table 5 shows that CLIA and ELISA gray-zone data mainly distributed in low titer of TPPA and high titer of TPPA, respectively. Our study indicated that ELISA had lower sensitivity and higher false-negative rate as a screening test compared with CLIA. Meanwhile, Table 4 shows that excluding the gray-zone data the sensitivity of ELISA could increase, and the sensitivity and specificity of CLIA were not influenced, which indicated that the data in ELISA gray zone should be paid more attention, and samples in ELISA gray zone had better be repeated with other treponemal test.

## CONCLUSIONS

Compared with ELISA, CLIA is more reliable, sensitive and accurate to detect serum *T. pallidum* specific

antibodies. In the future it may be an alternative test with higher sensitivity to ELISA as a screening test for syphilis diagnosis.

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## DISCLOSURE

The authors have declared no conflicts of interest.

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