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Evaluation of ELISA and CLIA for *Treponema pallidum* specific antibody detection in China: A multicenter study

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ABSTRACT

Background: Considering the rapid increase of syphilis infections in several countries including China, searching for a screening test with sufficient sensitivity and specificity is extremely urgent. The current study mainly researched the performance for *Treponema pallidum* (TP) detection by electro-chemiluminescence immunoassays (ECLIA), chemiluminescence immunoassays (CLIA) and four commercially available ELISA assays commonly used in China.

Methods: 1372 plasma samples collected from blood centers/banks were tested with 6 assays in 8 laboratories with the Western blot (WB) or TP particle agglutination assay (TPPA) as confirmatory tests.

Results: With the WB or TPPA as confirmatory test, the ECLIA demonstrated the highest specificity (95.2%) and Kappa coefficient (0.915), but lowest sensitivity (97.2%) compared with the other 5 assays. While the Wantai-ELISA showed the highest sensitivity (99.6%) among the 6 assays. Sensitivities were found to be significantly increased when any two of the six assays were combined for TP detection. Our study demonstrated that the Wantai-ELISA combined with the ECLIA or the KHB-ELISA or the InTec-ELISA would increase the sensitivities up to 100%. Further analysis showed that the specificities and positive predictive values were both 100.0% when cut-off of S/CO values were served as 15.42 for the ECLIA and 7.14 for the CLIA, indicating that samples under these conditions can be directly considered as positive without confirmation.

Conclusions: The CLIA and the ECLIA are more specific than ELISA to detect TP antibodies. However, ELISA is a sensitive method, especially in combination with the CLIA or the ECLIA or another types of ELISA, suitable for the routine screening of blood donations in China.

1. Introduction

Syphilis is a sexually transmitted infectious disease, which is caused by the spirochete *Treponema pallidum* subspecies *pallidum*, and is mainly spread *via* sexual exposure, mother-to-child transmission and blood transfusion(Peeling et al., 2017). In 2012, the WHO estimated that about 17.7 million individuals worldwide aged 15–49 years were infected with syphilis and 5.6 million new cases per year would occur globally(Meng et al., 2015). The estimated prevalence and incidence of syphilis vary in different regions and countries, with higher prevalence in developing countries(Elhadi et al., 2013).The infection rate of syphilis has increased dramatically in recent decades in China, becoming a serious public health concern(Zhang et al., 2016). The reported total syphilis incidence rate in China in the general population increased from 0.54 per 100,000 people in 1995 to 4.73 per 100,000 in 2000 and 26.86 and 32.86 per 100,000 populations in 2010 and in 2013, respectively(Zhang et al., 2016; Yang et al., 2017). Thus, early prediction of syphilis is therefore of great importance for health planning and management.

As *Treponema pallidum* can hardly be cultured *in vitro* or identified with simple laboratory stains(Edmondson et al., 2018), there is currently no gold standard test for identifying *T. pallidum*. Thus, serological tests naturally have become the most frequently used method for the diagnosis of syphilis both in clinical and in blood donor screening laboratories.

These serodiagnostic tests for syphilis can be broadly categorized

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into nontreponemal tests (NTTs) and treponemal tests (TTs). The Rapid Plasma Reagin (RPR) test, Toluidine Red Unheated Serum Test (TRUST) and Venereal Disease Research Laboratory (VDRL) test are the most commonly used NTTs, which are targeting the lipoidal components of this treponeme and are useful in detecting active syphilis(Ortel, 2012). As for the TTs, such as TP particle agglutination assay (TPPA), Western Blot (WB), fluorescent treponemal antibody (FTA), enzyme-linked immunosorbent assays (ELISA), chemiluminescence immunoassays (CLIA) and electrochemiluminescence immunoassays (ECLIA), they directly detect specific *T. pallidum* antibodies, which exist throughout whole life of infected patients. Although they are more specific than NTTs and are commonly used as confirmatory tests after a reactive NTT, they still cannot distinguish current or past infection and not useful in analyzing curative effect(Peeling et al., 2017).

China blood centers/banks takes the screening strategy of adopting two different *T. pallidum* antibodies (anti-TP) ELISA kits that are approved by the Chinese Food and Drug Administration for first-line screening(Ministry of Health, 2012). Dual ELISA reactive or non-reactive samples are defined as the final positive or negative donations, respectively. Discrepant samples would be retested in duplicate using the initially reactive ELISA kit and are considered to be (repeatedly) reactive if at least one of the repeat tests yielded positive results, and such samples, as well as the dual reactive samples, would be discarded for blood safety. The ECLIA and the CLIA, which have gradually been developed in recent years, can automatically and quickly detect serum or plasma *T. pallidum* specific antibodies with higher sensitivity and specificity. However, data about the performance affects comparison among commercially available ELISA, ECLIA and CLIA is still absent. The aim of this study was to investigate the detection performance of the ECLIA, the CLIA and four commercially available ELISA assays commonly used in China.

2. Materials and method

2.1. Samples

A total of 1372 plasma specimens, comprising 793 anti-TP reactive samples (showing dual-ELISA reactivity or repeat reactivity in routine blood bank procedures) and 579 anti-TP screening non-reactive samples (HBsAg, anti-HCV, anti-HIV and anti-TP all negative by both two screening EIAs in the enrolled laboratories, but discarded due to alanine transaminase (ALT) ≥ 50 U/L), were collected from healthy donors of blood banks in China from April 2015 to December 2015. Part of the blood donors' (n = 806) demographic characteristics were summarized in Table S1. All these plasma samples were stored at −20 °C prior to sending them to National Center for Clinical Laboratories (NCCL) and 8 independent laboratories for further testing for *T. pallidum* antibody.

2.2. Study design

8 independent laboratories in China were involved in this multi-center evaluation. All collected samples were tested with the CLIA, the ECLIA and 4 ELISA assays at the same time in various laboratories. Each ELISA assay was tested in at least 2 of the 8 laboratories. The ECLIA and the CLIA were performed in NCCL. Samples showing consistent results among all of these 6 assays were defined as either positive or negative. Discrepant results were firstly confirmed by TPPA, and the negative or indeterminate samples were further confirmed by WB. Any of these two tests with a positive result would be considered as positive(Sommese et al., 2016). Samples with an indeterminate outcome for TPPA or WB were excluded from the interassay comparisons.

2.3. Assays

2.3.1. ELISA tests

The four different commercially available anti-TP assays that are the most widely used ELISAs in China, include the InTec-ELISA (InTec Products, Xiamen, China) and KHB-ELISA (Shanghai Kehua Bioengineering, Shanghai, China), Wantai-ELISA (Beijing Wantai Biological Pharmacy, Beijing Wantai, China) and Livzon-ELISA (LivzonDiagnostics Inc., Zhuhai, China). All kits are two-step double-antigen sandwich (DAGS) ELISA tests where the plates have been coated with the recombinant *T. pallidum* antigens (TpN15, TpN17, and TpN47) and were performed following the manufacturer's instructions.

ELISA's results of each sample: one sample tested by the same assay showed consistent results in different laboratories, the assay's result of this sample was the same as the consistent result. When different stations showed different results, the majority result was taken as the assay's result of this sample. When the number of positives equaled negatives, then the average signal to cutoff value (S/CO) was taken as the assay's result.

2.3.2. Chemiluminescence immunoassays (CLIA) and electrochemiluminescence immunoassays (ECLIA)

All of these samples were also tested with the CLIA and the ECLIA at NCCL. The CLIA was performed using the Architect syphilis TP system (Abbott, Wiesbaden, Germany), which is a qualitative method by incubation of the serum with the paramagnetic microparticles coated with recombinant TpN15, TpN17, and TpN47 antigens, followed by addition of acridinium-labeled anti-human IgG or IgM conjugates and measurement of chemiluminescence reaction as relative light units (RLUs), which is directly related to the amount of anti-*T. pallidum* antibodies in the sample. RLU of specimen/cut-off (S/CO) ratio ≥ 1 was scored positive.

The ECLIA (Roche Diagnostics, Mannheim, Germany) was performed using the Cobas e601 platform. The automated Elecsys immunoassay is a one-step DAGS assay. A mixture of biotin- and ruthenium-labeled recombinant TpN15, TpN17, and TpN47 antigens were incubated with sample to form a DAGS immune complex, followed by addition of streptavidin-coated paramagnetic microparticles. The results were expressed as a S/CO ratio calculated by the analyzer software, with a S/CO of ≥1.0 indicating a reactive result.

2.3.3. Confirmatory tests

Discrepant plasma samples (reactive with at least one but not all of 6 assays) were all firstly subjected to confirmatory testing with the Serodia TPPA (Fujirebio, Tokyo, Japan). Negative and indeterminate results conducted by TPPA were further confirmed by Western blot (Mikrogen Diagnostic, Martinsried, Germany). All results were performed according to the manufacturer's instructions. Any of these two confirmatory tests with a positive result would be considered as positive. Both tests with negative results were referred as being negative. Immunoblot assay used nitrocellulose membrane strips fixed with the recombinantly-produced antigens: Tp47, TmpA, Tp257(Gpd), Tp453, Tp17 and Tp15. The strips were firstly incubated with 20-μL plasma samples and then with the horseradish-peroxidase-labeled secondary antibodies specific for human IgG or IgM. The reactivity of each antigen band was visualized after the addition of 3,3′,5,5′-Tetramethylbenzidine (TMB) and was assessed by comparison with the intensity of the cut-off control band. At least 2 of the 6 bands antigen equivalent or stronger than cutoff band was interpreted as “positive” and only one random band antigen equivalent or stronger than cutoff band was interpreted as “indeterminate” (Tao et al., 2017).

The TPPA uses gelatin particle carriers sensitized with purified *T. pallidum* (Nichols strain). Definite large and rough outer margins appearing on the bottom of the microtiter plate well large were interpreted as “positive”, particles compacted on the button of the plate well as “negative”, and particles forming a smooth round outer margin concentrated in the shape of a compact as “indeterminate” according to the instructions of the manufacturer.

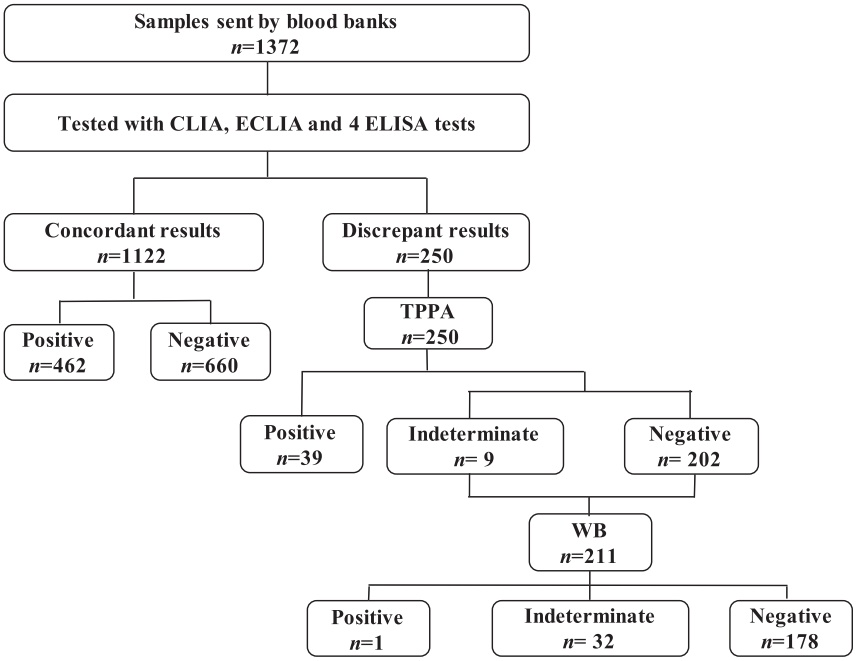

Fig. 1. The testing algorithms of syphilis in our study.
TPPA, *T. pallidum* particle agglutinationassay; WB, Western blot; ELISA, enzyme-linked immunosorbent assays, CLIA, Chemiluminescence immunoassays; ECLIA, electrochemiluminescence immunoassays.

Table 1
Sensitivity, specificity and Kappa coefficient of six evaluated assays and different combinations with TPPA/WB as confirmatory tests.[a]

Testing methods and parameters[a]	ECLIA	CLIA	KHB	InTec	Livzon	WANTAI
Sensitivity % (95% CI)						
ECLIA	97.2 (95.4–98.5)					
CLIA	98.0 (96.4–99.0)	92.2 (89.5–94.4)				
KHB	99.6 (98.6–100.0)	99.6 (98.6–100.0)	99.4 (98.3–99.9)			
InTec	99.2 (98.0–99.8)	99.4 (98.3–99.9)	99.8 (98.9–100.0)	99.0 (97.7–99.7)		
Livzon	98.4 (96.9–99.3)	98.6 (97.2–99.4)	99.6 (98.6–100.0)	99.0 (97.7–99.7)	97.8 (96.1–98.9)	
WANTAI	100.0[b] (99.3–100)	99.6 (98.6–100)	100.0[b] (99.3–100.0)	100.0[b] (99.3–100.0)	99.8 (98.9–100.0)	99.6[c] (98.6–100.0)
Specificity % (95% CI)						
ECLIA	95.2 (93.6–96.6)					
CLIA	94.1[b] (92.4–95.6)	98.0[c] (96.9–98.8)				
KHB	86.7 (84.2–88.9)	86.7 (84.2–88.9)	88.1 (85.7–90.2)			
InTec	88.8 (86.5–90.9)	88.2 (85.9–90.3)	84.9 (82.3–87.2)	89.5 (87.3–91.5)		
Livzon	92.3 (90.3–94.0)	91.8 (89.7–93.5)	84.9 (82.3–87.2)	87.6 (85.2–89.8)	93.0 (91.0–94.6)	
WANTAI	87.6 (85.2–89.8)	87.8 (85.9–89.9)	83.1 (80.4–85.6)	84.9 (82.3–87.3)	86.4 (83.9–88.7)	89.1 (86.8–91.1)
Kappa value						
ECLIA	0.915[c]					
CLIA	0.906[b]	0.910				
KHB	0.826	0.826	0.843			
InTec	0.833	0.844	0.805	0.857		
Livzon	0.886	0.882	0.803	0.833	0.890	
WANTAI	0.841	0.840	0.786	0.808	0.825	0.856

CLIA, Chemiluminescence immunoassays; ECLIA, electrochemiluminescence immunoassay.
[a] Confirmed indeterminate samples were excluded from the specificity assessment.
[b] The highest specificities, sensitivities and Kappa value among different combinations.
[c] The highest specificities, sensitivities and Kappa value among 6 assays.

2.4. Statistical analysis

The sensitivity, specificity and Kappa values were calculated by SPSS software (V.21.0). We evaluated 6 TP assays for analytical sensitivity and specificity and for consistency with the confirmatory test by Kappa coefficients. Poor consistency was given for Kappa coefficients of between 0.00 and 0.20; fair (0.21 to 0.40); moderate (0.41–0.60); good (0.61–0.80) and very good (0.81–1.00).

3. Results

3.1. Comparison of the consistency, sensitivity, and specificity among six detection assays as well as in different combinations

Of the 1372 samples that were tested, 250 samples showed discrepant results and 1122 plasma samples showed consistent results among the six assays. In the 1122 consistent samples, 462 were identified as positive and 660 were negative (Fig. 1). In total, there were 502 true positive results, 838 true negative results and 32

indeterminate results when the WB and TPPA were used as the confirmatory tests. Firstly, we calculated the consistency, sensitivity, and specificity of the ECLIA, the CLIA and four ELISA assays compared with the confirmatory tests. Consistency analysis showed that Kappa coefficient between ECLIA and confirmatory test (Kappa = 0.915) was higher than that between any ELISA or CLIA test and confirmatory test (range 0.843–0.910). The Livzon-ELISA showed the highest Kappa coefficient (0.890) among various ELISA tests. All assays demonstrated a high coefficient of correlation (0.81 to 1.0). The results of interassay comparisons for the detected samples revealed that the sensitivity was highest for the Wantai-ELISA test(99.6%)and lowest for the Roche ECLIA test (97.2%). The CLIA immunoassay demonstrated the highest specificity (98.0%) compared with other 5 assays and the Livzon-ELISA showed the highest specificity (93.0%) among these 4 ELISA assays.

We combined any two of the 6 assays to evaluate whether these combinations would increase the detection efficiency, using the WB/TPPA as confirmatory tests. For this combination, we referred to one or both tests with a positive result as “positive” and both with negative results as “negative”. As defined above, combinations of any two assays showed increased sensitivity (range 98.0%~100%) compared with the corresponding tests considered in isolation (Table 1). Nevertheless, the improved sensitivity was at the cost of specificities, which ranged from 83.1% to 94.1% (Table 1). The sensitivities achieved 100.0% when the Wantai-ELISA was combined with the ECLIA, the CLIA or the Livzon-ELISA. However, the specificity and Kappa coefficient were lower than any assay in each combination. In addition, the ECLIA combined with CLIA showed the highest specificity (94.1%) and Kappa coefficient (0.906) compared to any other combinations (Tables 2 and 3).

3.2. Correlation between S/CO values of ECLIA or CLIA and confirmatory results (WB/TPPA)

We analyzed samples by their S/CO values and the confirmatory results to investigate whether there is an interconnection between them. By the ECLIA, 381 (99.74%) of 382 samples (S/CO > 10), 419 (99.52%) of 420 samples (S/CO > 6) and 462 (90.69%) of 474 samples (S/CO > 3) were confirmed by TPPA/WB reactivity, respectively. As for the CLIA test, 100% (323/323), 99.75% (392/393) and 98.08% (458/471) samples were TPPA/WB positive with S/CO > 6, S/CO > 3 and S/CO > 1. Analysis the receiver operating characteristic curve (ROC) for the two assays indicated that increasing the cut-off to a 15.42 S/CO value for the ECLIA and 7.14 for the CLIA (data not shown) would increase both of the specificities and positive predictive value (PPV) to 100%.

Table 2
Correlation between S/CO values of ECLIA or CLIA and confirmatory results (WB/TPPA).

Tests	S/CO	Totally tested No.	No. with WB or TPPA result of:			Positive rate (%)
			Positive	Indeterminate	Negative	
ECLIA	> 10	382	381	0	1	99.74
	> 6	38	37	1	0	99.52
	> 3	54	43	3	8	97.25
	> 1	63	26	6	31	90.69
CLIA	> 10	276	276	0	0	100.00
	> 6	47	47	0	0	100.00
	> 3	70	69	0	1	99.75
	> 1	74	66	2	6	98.07

TPPA, *T. pallidum* particle agglutinationassay; WB, Western blot; CLIA, Chemiluminescence immunoassays; ECLIA, electrochemiluminescence immunoassays.

3.3. Characterization of indeterminate results that were confirmed by Western blot

Of the 32 samples which were finally confirmed as indeterminate, 27 samples were WB indeterminate but TPPA negative, 3 samples were TPPA indeterminate but WB negative, and 2 samples confirmed as being indeterminate by both tests. We analyzed the distributions of seven antigens of the 29 WB confirmed indeterminate samples. It revealed that 18 (62.07%) samples were IgM reactive (including 8 IgM TpN47 reactive, 7 IgM TpN15 reactive and 3 IgM TpN17 reactive samples) and 11 (37.93%) samples were IgG reactive (including 6 IgG TpN47 reactive, 2 IgG TpN15 reactive, 2 IgG TpN17 reactive and 1 IgG TpN257 reactive samples). All of these samples were classified into 4 patterns according to the CLIA and ECLIA outcomes. 1 sample was CLIA + /ECLIA + , 9 samples were CLIA-/ECLIA + , 2 samples were CLIA + /ECLIA- and 17 samples were CLIA-/ECLIA-. We found that all of the IgM TpN15 reactive samples were CLIA-/ECLIA- and 7/8 IgM TpN47 reactive samples were CLIA-/ECLIA-. In the IgG TpN47 reactive samples, 4/6 were CLIA-/ECLIA + and 2/6 were CLIA + /ECLIA-.

4. Discussion

Serological screening has been the main approach for confirming the diagnosis of suspected cases of syphilis infection. However, due to different reagents suppliers, different sources and concentrations or ratios of coated or labeled recombinant antigens, while showing variability in the sensitivities of detection reagents, so the sensitivity and specificity is difference from different reagent manufacturers and different methods. The present study established that most ELISA tests demonstrated higher sensitivities compared with the ECLIA and the CLIA. In contrast, the ECLIA and the CLIA showed higher specificities and consistency with the confirmatory test than that of the ELISA tests. However, the sensitivities, specificities and consistencies of the ECLIA and the CLIA evaluated in this study do not support the previous research studies. Park and colleagues compared the performance of six assays including the ECLIA and the CLIA for the detection of TP antibodies with the FTA-ABS as the confirmatory method and reported higher sensitivities and specificities and Kappa values (Park et al., 2016), than those in our study. Another study demonstrated that the ECLIA assay displays a better sensitivity (100%) and specificity (99.8%) compared with the InTec assay (99.6% and 99.7%) and the KHB assay (98.56% and 99.77%) in 999 previously confirmed syphilis cases (Antonella et al., 2013). The study of Li et al., also displayed similar detection performance of the ECLIA and the InTec assay, but also demonstrated different specificities (97.4–100.0%) in different populations, such as infected patients, clinical routine samples and potential cross-reactive samples (Li et al., 2016a, 2016b), indicating different detection performance among various population subgroups. All of the ELISA kits demonstrated similar sensitivities compared with previous studies, but the specificities were much lower than that reported in these studies (Yang et al., 2017; Tao et al., 2017). An important reason to explain it is because that the enrolled samples in our study were different from other studies. Though our specimens were obtained from healthy blood donors, they were primarily screened by commercially available ELISA assays mentioned above. Thus, there may be more false-positives and false-negatives in these primary reactive samples than other populations such as individuals with clinically confirmed syphilis. In addition, different reference tests may be a possible explanation for diverse detection performance. Jonckheere and coworkers evaluated the TPPA and another six assays (eg. WB) as possible confirmatory assays in CLIA-positive and RPR-negative samples (Jonckheere et al., 2015). The overall agreement between the TPPA and the six alternative tests ranged from 44.6% to 82.0%, showing a poor agreement among the confirmatory tests in this group (Jonckheere et al., 2015). Though TPPA is a recommended confirmatory test in many laboratories, with adequate sensitivity and specificity, the TPPA

Table 3
Characterization of indeterminate results confirmed by Western Blot.

Pattern	Number	IgG				IgM		
		TpN17	TpN47	TpN15	TpN257	TpN15	TpN17	TpN47
CLIA + /ECLIA +	1	1	0	0	0	0	0	0
CLIA-/ECLIA +	9	1	4	1	1	0	1	1
CLIA + /ECLIA-	2	0	2	0	0	0	0	0
CLIA-/ECLIA-	17	0	0	1	0	7	2	7
Total	29	2	6	2	1	7	3	8

TPPA, *T. pallidum* particle agglutinationassay; CLIA, Chemiluminescence immunoassays; ECLIA, electrochemiluminescence immunoassays ; +: positive; −:negative; ± : indeterminate.

is subject to individual variations in interpretation and has been reported to show lower sensitivity than WB. Thus, for security reasons, TPPA negative and indeterminate samples were re-tested with WB in our study. As the discrepant samples were reactive in at least one of the six tests in our study, such set of samples were more likely to be positive if they were confirmed positive by any one of the two confirmatory assays. The consistent samples among the 6 assays without further confirmation was partly to save money, but there is no gold-standard test to adjudicate these results, so we could not provide any guarantee for the accuracy of the TPPA or WB when they showed contrary results in all of the 6 assays. In addition, diverse detection performance might also be related to the different disease stages, high or low prevalence areas, the field or laboratory test conditions as well as possible methodological modifications (Marangoni and Paola Nardini, 2013; Centers for Disease Control and Prevention, 2011; Bazzo et al., 2017). These parameters should be analyzed in further studies.

A strategy for TP antibody testing using two enzyme immunoassay kits for routine blood screening program in China has been applied since 2012, but the sensitivity and specificity of combinations of different enzyme immunoassays had not been evaluated. In this study, we compared the detection performance of different combinations. It is well known that, sensitivities of serological tests are always negatively correlated with the specificities. As expected, the diagnostic sensitivity reached 100% when the Wantai-ELISA was combined with the ECLIA or the KHB-ELISA or the InTec-ELISA, but the specificities were decreased. Otherwise, although the ECLIA and the CLIA are two efficient detection methods commonly used as first line for blood screening in most developed countries, combination of these two assays was not sensitive enough to identify the true-positives. If one sample was judged positive, the donation was discarded and the donor was permanently deferred in China (Ministry of Health, 2012). Thus, the combination strategy has increased the sensitivity to exclude blood donors from future donations for safety reasons. But at the same time, the increased false positive rates, leading to unnecessary disposal of healthy blood as well as intangible loss with respect to donors, particularly repeat donors, cannot be ignored. Therefore, confirmatory tests, such as TPPA and WB, with high specificities should be employed to rule out false-positive reactions. Based on our findings, we recommend that, given the number of infectious syphilis cases, 100% sensitivity conducted by the dual-tests followed by an additional confirmatory test to avoid false-positives was reasonable in blood screening. However, TPPA or WB based confirmation appears expensive from a blood station standpoint when compared with traditional dual-kits-based screening. Thus, it is crucial in creating guidelines for the use of *Treponema* confirmation tests in syphilis screening.

In this study, we found that higher S/CO values for the CLIA and the ECLIA correlated with higher diagnostic reliability. ROC analysis indicated that a cut-off using S/CO of 7.14 on Architect Syphilis TP assay showed 100% specificity and the PPV, indicating an excellent predictive ability. This result accords with earlier observations, which reported that over 90% samples were TPPA-positive with the cut-off of S/CO value above 6.00 (Li et al., 2016b). Another study suggested that increasing the cut-off of the CLIA to 5.6 S/CO resulted in a comparable diagnostic performance (Jonckheere et al., 2015). Lee and colleagues found that the CLIA showed highest efficacy when cut-off using S/CO of 3.1 (sensitivity 82.7%, specificity 87.5%) in the CIA (+)/RPR (−) sera (Lee et al., 2013). Regarding the Roche ECLIA with S/CO above 6.00, we found that 99.52% (418/420) samples were confirmed positive. Besides, the specificity and PPV were both increased to 100% when the cut-off of the ECLIA increased to a value of 15. 42 S/CO. This result is much higher than the findings of Sommese et al., which showed that serum with S/CO above 8.00 by the Roche ECLIA were frankly positives (Sommese et al., 2016). If the false-positive sample with the high S/CO value for 15.42 detected by the Roche ECLIA was excluded from calculations, all of those samples were true positives when S/CO was higher than 5.88, showing 100% specificity and PPV. Thus, according to the findings in our study, and taking the cost into consideration, we can infer that all samples with S/CO over 5.88 by the Roche ECLIA or 7.14 by the Architect CLIA are rarely negative and, can be directly reported as positive without confirmation, which could decrease the demand and the cost of a confirmatory test.

Among the 29 WB indeterminate results, 62.1% samples were IgM reactive. This may be due to the fact that IgM is produced earlier than IgG antibodies and the levels of IgM may be too low to be detected by certain assays. Moreover, IgM antibodies are often produced as a result of a non-specific activation of the immunological response (Jonckheere et al., 2015). WB may be more sensitive than CLIA, ECLIA and ELISA to detect IgM antibodies. This difference may be potentially due to less than optimal concentrations and ratios of the specific *T. pallidum* antigens are being used in the anti-TP ELISA or CLIA method (Wang and Li, 2009). As for IgG, we found more than half of the samples showed reactivity to TpN47, probably because IgG TpN47 was often more intense in primary syphilis. Hanff et al. observed that infected patients already showed TpN47 and TpN37 reactivity in the previous 3 to 6 days (Hanff et al., 1983). Norris et al. found that even the healthy individuals contain IgG antibodies against the TpN47 fraction (Norris, 1993). However in the Sommese et al. study, TpN47 presented in none of 3 indeterminate samples that were confirmed by INNO-LIA, while 2 were reactive against TpN17, one was reactive against TpN15 (Sommese et al., 2016). Another study showed variable antigen reactivity patterns by 4 different antigen-specific assays in 74 CLIA-positive/RPR-negative/TPPA-negative samples (Jonckheere et al., 2015). Thus, different reactivity patterns were probably due to the different indeterminate samples size and various confirmatory methods used in studies. However, whether these indeterminate results indicate a non-specific reaction or an early infection is still uncertain. Further study or follow-up tests that attempt to combine clinical and demographic information are needed to evaluate the indeterminate results depending on with an enlarged number of samples in this particular population.

In conclusion, the CLIA and the ECLIA assays represent a higher detection specificity than the other 4 ELISA tests widely used in China. But their false-positive results cannot be ignored, especially for the S/CO values lower than 6.0 for the Roche ECLIA and 3.0 for the Architect CLIA. In addition, ELISA is a sensitive method, especially in

combination with the CLIA or the ECLIA or another ELISA assays, suitable for the routine screening of blood donations, but further confirmation is recommended to rule out false-positive reactions.

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Conflicts of Interest and Source of Funding

None.\

Appendix A. Supplementary data

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