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Clinical application value of Abbott Alinity analyzer in syphilis-specific antibody testing

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ABSTRACT

Background: We aimed to investigate the clinical application value of Abbott Alinity analyzer in syphilis-specific antibody testing.

Methods: A total of 100 patients admitted from June 2021 to June 2022 for early syphilis diagnosis were selected and subjected to Treponema pallidum (TP) antibody testing by chemiluminescent microparticle immunoassay (CMIA) using Abbott Alinity analyzer. With TP particle agglutination (TPPA) retesting as the gold standard for syphilis diagnosis, the signal-to-cutoff (S/CO) ratio of the TP antibody testing was plotted into the receiver operating characteristic curve to determine the diagnostic value of CMIA and CLIA in detecting positive TP antibody and to identify the optimal cutoff point.

Results: In the case of S/CO ratio ≥7.00, the patients with positive CMIA were diagnosed with positive findings after TPPA confirmation. With the S/CO ratio of 1.00-4.99, the coincidence rate of CLIA with positive TPPA was 81.82% (45/55), and all patients with positive CLIA had positive results confirmed by TPPA test when the S/CO ratio was >5.00. When the optimal cutoff value of S/CO ratio for TP was determined as 6.98 by CMIA, the sensitivity, specificity, and maximum area under the curve (AUC) were 94%, 88% and 0.91, respectively. At the optimal cutoff value (S/CO ratio: 4.56) determined by CLIA, the sensitivity was 84%, the specificity was 80%, and the maximum AUC was 0.84.

Conclusions: In the case of S/CO ratio ≥7.00, both methods have high sensitivity and specificity, which can directly give positive reports and shorten the sample turnaround time.

Keywords: Abbott, Alinity analyzer, cutoff point, diagnosis, syphilis-specific antibody

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INTRODUCTION

Syphilis is a chronic sexually transmitted disease caused by ***Treponema pallidum*** (TP) infection of human body, which is a global health problem. In China, the incidence rate of syphilis was raised by over three times during 2005-2014 with the accelerated economic development and population migration, and its increase was greater than other infectious diseases in the surveillance system (1). In 2019, 535,819 new cases of syphilis were reported in China (2). As a species of the ***Treponema*** genus, TP is also known as ***Spirochaeta pallida*** due to its transparent and non-staining characteristics. It can cause damage to multiple system organs after infecting the human body, resulting in clinical manifestations such as hard chancre and systemic rash and eventually inducing tissue destruction and malfunction. Besides, it may be life-threatening as the disease progresses (3). Given the lack of efficacious vaccines, the control of syphilis transmission largely depends on timely and accurate diagnosis, so as to provide patients with prompt early treatment and eliminate the source of infection (4). Some patients have obscure or even no clinical symptoms, which increases the difficulty of clinical diagnosis. Therefore, it is particularly important to conduct laboratory diagnosis in order to improve the timeliness and accuracy of diagnosis (5,6).

The traditional laboratory diagnosis of syphilis is usually based on serological screening, including rapid plasma reconstitution and toluidine red unheated serum test. Serological experiment remains the preferred method for clinical diagnosis of TP and monitoring of subsequent antibiotic therapeutic effects at present due to the difficulty of ***in vitro*** culture of TP (7). The diagnosis can be further confirmed by TP antibody specificity tests like TPPA test and fluorescent triad antibody absorption test or enzyme-linked immunosorbent assay (8). However, fluorescent triad antibody absorption test or enzyme-linked immunosorbent assay poses extremely high requirements on equipment and personnel, such as refrigeration for storing reagents, centrifugation for separating serum, electric rotator for operating flocculation

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tests and experienced laboratory operators, which are generally insuperable barriers to testing in developing countries and grassroots hospitals with scarce medical and health resources (9). TPPA test is an official method used in the United States to confirm the diagnosis, which is also an accurate serological test for syphilis patients generally accepted worldwide. It mainly detects TP-specific antibody and serves as an important testing method to determine whether a patient has been infected with syphilis (10,11). This method is sensitive, accurate, and insusceptible to biological factors, but it has such shortcomings as great difficulty in operation technology, many steps and long testing time because the TP strains need to be prepared into antigen first and then wrapped on and closely combined with the sensitized particles, thus generating agglutinated particles. Meanwhile, chemiluminescent microparticle immunoassay (CMIA) has been widely applied in clinical practice because of its full automation and high diagnostic efficiency. However, the excessively high sensitivity of CMIA may cause false positive results (12).

On this basis, the diagnostic value of Alinity analyzer, a new-generation ***in vitro*** diagnostic product of Abbott, in TP was investigated, whether some complementary experiments need to be implemented to increase the early diagnostic accuracy of TP infection was analyzed, and a reasonable retest threshold of signal-to-cutoff (S/CO) ratio was determined to reduce the false-positive reporting rate of CMIA using Abbott Alinity analyzer in this study.

MATERIALS AND METHODS

General data

A total of 100 patients admitted to Yuyao People's Hospital of Zhejiang Province from June 2021 to June 2022 for early syphilis diagnosis were selected. This study was approved by the ethics committee of our hospital. There were 62 males and 38 females aged 22-68 years old and (52±7) years old on average, most of whom were in the age group of 41-59 year-olds. The inclusion criteria were set as follows: 1) Patients who had positive silver staining results (negative diplococci) and met clinical diagnostic criteria for syphilis, 2) those with an age of no less than 20 years old and no more than 70 years old, and 3) those who signed the informed consent to relevant operations. The exclusion criteria included 1) patients complicated with serious injury of the heart, liver, kidney or other vital organs, those with systemic immune diseases, mental diseases, positive HIV or sensitivity to penicillin, or 3) those who recently received antibiotic treatment.

Sample collection

Fasting venous blood (4 mL) was collected from all patients in the early morning and then added to anticoagulant tubes containing EDTA and sterile collection tubes containing separating gel. Afterwards, the tubes were centrifuged at 3500 r/min (radius: 25 cm) for 5 min. The serum was collected and stored in a -20°C refrigerator.

Detection methods

All patients underwent chemiluminescence immunoassay (CLIA), CMIA using Abbott Alinity analyzer and TP particle agglutination (TPPA) test. CLIA and CMIA using Abbott Alinity analyzer were the testing methods for syphilis-specific antibody, while TPPA test was mainly used for retesting.

CLIA method: CLIA was conducted by using 2 NCU/mL anti-syphilis quality control serum provided by the National Center for Clinical Laboratories (China) and HISCL-800 CLIA analyzer (Sysmex, Japan).

CMIA method: Alinity analyzer (Abbott, USA) and supporting reagents, calibration solutions, and quality controls were used.

TPPA test method: TPPA kit was purchased from Fujirebio (Japan), and the results were determined by reference to the following criteria: With the cell deposition state in the center of reaction wells on the culture plate as the judgment basis, the scattered aggregation of cell deposition was determined as positive, while the smooth button-like cell deposition was determined as negative. The above test procedures were implemented in strict accordance with the operation instructions.

Observation and evaluation indexes

(1) In terms of the judgment criteria, the results were determined positive if the signal-to-cutoff (S/CO) ratio ≥1.0 was output by the instruments of CMIA and CLIA, and they were negative if the S/CO ratio <1.0 was obtained. In TPPA test, the results would be judged as positive if there was obvious irregularity of agglutination in the sensitized particle wells. The results of different testing methods for all samples were completely recorded.

(2) The results of CMIA and CLIA were segmented and ranked by S/CO ratios, which were divided into 6 groups to calculate the coincidence rates of CMIA and CLIA results with positive TPPA. Moreover, receiver operating characteristic (ROC) curves were plotted with the S/CO ratios of CMIA and CLIA, and the area under the curve (AUC) was calculated, thus obtaining the optimal cutoff values for the diagnostic efficiency of the two methods in TP.

Statistical analysis

SPSS 24.0 software was employed for statistical analysis. The count data were expressed by percentage (%) using the χ^2 test, and the diagnostic values of the two methods for TP were explored by plotting ROC curves. P<0.05 suggested a statistically significant difference.

RESULTS

General data of subjects

Among the 100 serum samples, there were 73 confirmed cases of positive TPPA. The age range of the patients was 21-68 years old, and the majority (64%) of the patients were aged between 41 and 59 years old. Besides, 62 (62%) subjects were male and 38 (38%) were female. Most (67, 67%) subjects resided in nearby rural areas and 33 (33%) were urban residents (**Table 1**).

Diagnostic efficiency of CLIA and CMIA for early syphilis

Of the 100 serum samples, there were 76, 83, and 73 cases of positive CMIA, positive CLIA, and positive TPPA, respectively. With TPPA test as the confirmation method, the sensitivity of CMIA was 95.89%, the specificity was 77.78%, the false negative rate was 4.11%, the false positive rate was 22.22%, and the diagnostic accuracy for early syphilis was 91.00%. The sensitivity, specificity, false negative rate, false positive rate, and diagnostic accuracy for early syphilis of CLIA was 94.52%, 48.15%, 5.48%, 51.85%, and 82.00%, respectively. In short, CMIA had significantly higher specificity and accuracy and lower false negative or positive rate than those of CLIA (P<0.05) (**Table 2**).

Coincidence rate of positive prescreening by CMIA and CLIA with TPPA confirmation for TP antibody in different ranges of S/CO ratio

The S/CO ratio of the 100 samples was within the range of 1.00-6.99, and the coincidence rate of positive TPPA with CMIA using Abbott Alinity analyzer was 89.29% (25/28). When the S/CO ratio was ≥7.00, the results of the patients with positive CMIA were confirmed positive by TPPA test. At the S/CO ratio of 1.00-4.99, the coincidence rate of positive TPPA with CLIA was 81.82% (45/55). In the case of the S/CO ratio >5.00, all patients with positive CLIA had positive results after TPPA confirmation (**Tables 3 and 4**).

Table 1. General data of subjects [n (%)]

Demography		Total	Positive TPPA (%)	Negative TPPA (%)	P
Sex	Male	62	46 (74.19%)	16 (25.81%)	P>0.05
	Female	38	27 (71.05%)	11 (28.95%)	
Age (Y)	22-40	23	17 (73.91%)	6 (26.09%)	P>0.05
	41-59	64	46 (71.88%)	18 (28.13%)	
	≥59	13	10 (76.92%)	3 (23.08%)	
Residence	Urban	33	20 (60.61%)	13 (39.39%)	P>0.05
	Rural	67	53 (79.10%)	14 (20.90%)	

Table 2. Sensitivity, specificity, false negative rate, false positive rate, and accuracy of the two testing methods [n (%)]

Testing method	Sensitivity	Specificity	False negative rate	False positive rate	Accuracy
CMIA	95.89(70/73)	77.78(21/27)	4.11(3/73)	22.22(6/27)	91.00(91/100)
CLIA	94.52(69/73)	48.15(13/27)	5.48(4/73)	51.85(14/27)	82.00(82/100)
X2	0.150	5.082	0.150	5.082	4.153
P	0.699	0.024	0.699	0.024	0.042

Table 3. Coincidence rate of positive prescreening by Abbott Alinity analyzer with TPPA confirmation for TP antibody in different ranges of S/CO ratio

Range of S/CO ratio	Number of prescreened cases by Abbott Alinity analyzer	Number of true positive TPPA	Number of negative TPPA	Coincidence rate with positive TPPA (%)
1.00~3.99	16	14	2	87.50
4.00~6.99	12	11	1	91.67
7.00~9.99	10	10	0	100.00
10.00~12.99	6	6	0	100.00
13.00~15.99	10	10	0	100.00
≥16.00	22	22	0	100.00
Total	76	73	27	97.33

Table 4. Coincidence rate of positive prescreening by CLIA with TPPA confirmation for TP antibody in different ranges of S/CO ratio

Range of S/CO ratio	Number of prescreened cases by CLIA	Number of true positive TPPA	Number of negative TPPA	Coincidence rate with TPPA positive (%)
1.00~1.99	26	21	5	80.77
2.00~2.99	11	10	1	90.91
3.00~3.99	7	6	1	85.71
4.00~4.99	11	10	1	90.91
5.00~5.99	9	9	0	100.00
≥6.00	19	19	0	100.00
Total	83	73	27	87.95

Table 5. Diagnostic value of CMIA using Abbott Alinity analyzer for TP antibody through ROC curve analysis

Group	Cutoff value	Sensitivity (%)	Specificity (%)	AUC	95% CI
CMIA	6.98	94%	88%	0.91	0.826-0.941
CLIA	4.56	84%	80%	0.84	0.692-0.898

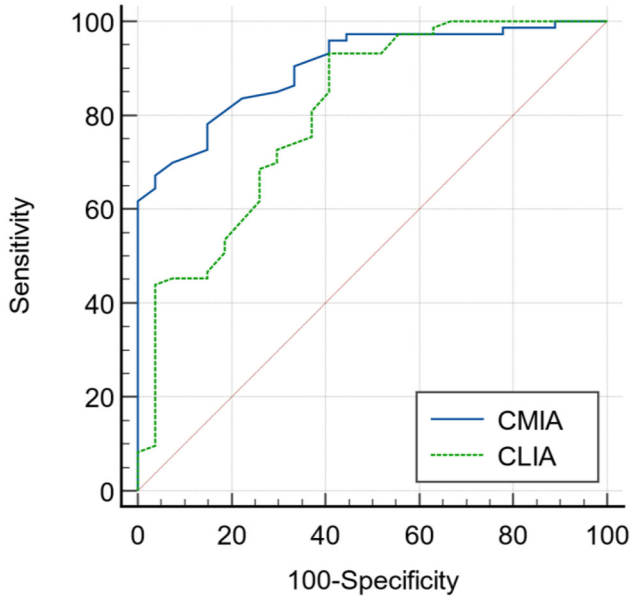

Fig. 1. Diagnostic values of CMIA and CLIA for TP antibody.

Diagnostic value of CMIA using Abbott Alinity analyzer for TP antibody through ROC curve analysis

The results of ROC curve analysis exhibited that when the optimal cutoff value of S/CO ratio for TP was determined as 6.98 by CMIA, the sensitivity, specificity and maximum AUC were 94%, 88%, and 0.91, respectively. At the optimal cutoff value (S/CO ratio: 4.56) determined by CLIA, the sensitivity was 84%, the specificity was 80%, and the maximum AUC was 0.84 (**Table 5** and **Figure 1**).

DISCUSSION

In this study, with TPPA test as the confirmation method for TP antibody, the diagnostic efficiency of CMIA using Abbott Alinity Analyzer and CLIA in diagnosing TP was compared. The results showed that both CMIA and CLIA possessed high sensitivity. The specificity and accuracy of CMIA significantly increased compared with those of CLIA, while the false negative or positive rate reduced ($P<0.05$), because Abbott Alinity analyzer had various advantages (13,14).

The high sensitivity of CMIA in detecting TP antibody may lead to false positive results using Abbott Alinity analyzer (15,16). Likewise, we herein found that the S/CO ratio of the 100 samples was within the range of 1.00-6.99, and the coincidence rate of positive TPPA with CMIA using Abbott Alinity analyzer was 89.29% (25/28). When the S/CO ratio was ≥7.00, the results of the patients with positive CMIA were confirmed positive by TPPA test. At the S/CO ratio of 1.00-4.99, the coincidence rate of positive TPPA with CLIA was 81.82% (45/55). In the case of the S/CO ratio >5.00, all patients with positive CLIA had positive results after TPPA confirmation. Li ***et al***. reported that there was false positivity at the S/CO ratio of 1-10 using Architect i2000 analyzer (Abbott, USA) (17). In order to avoid incorrect diagnosis, confirmatory tests are required for serum samples with the S/CO ratio of CMIA in a low value range. Therefore, the mode of CMIA prescreening combined with TPPA retest is usually employed to confirm the diagnosis of TP antibody.

Nevertheless, determining the retest threshold of CMIA is challenging. In the present study, the ROC curve was plotted to explore the optimal threshold of CMIA using Abbott Alinity analyzer for syphilis screening. At the optimal cutoff value (S/CO ratio: 6.98) determined by CMIA, the sensitivity was 94%, the specificity was 88%, and the maximum AUC was 0.91. At the optimal cutoff value (S/CO ratio: 4.56) determined by CLIA, the sensitivity was 84%, the specificity was 80%, and the maximum

AUC was 0.84. Hence, the diagnostic efficiency of CMIA using Abbott Alinity analyzer for detecting TP antibody was superior to that of CLIA. In terms of CMIA for detecting TP antibody, when the S/CO ratio was ≥7.00, the positive results can be directly used for issuing positive reports of syphilis due to the preferable diagnostic efficiency. For the results with the S/CO ratio <6.99, further TPPA test is recommended to avoid false positive reports. In the case of negative results, TPPA test should be performed again. This strategy can provide reliable results for the rapid clinical detection of TP antibody.

In conclusion, both CMIA using Abbott Alinity analyzer and CLIA have good diagnostic performance for syphilis. When the S/CO ratio is ≤6.99, CMIA using Abbott Alinity analyzer has a certain false positive rate, and TPPA rest must be conducted to ensure the accuracy of the test results. At the S/CO ratio of ≥7.00, both methods have high sensitivity and specificity, positive reports can be directly issued, thus shortening the sample turnaround time. However, this study still had limitations, such as small sample size, and the results should be validated by larger sample sizes in future studies.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTION

SH- Study design, Data analysis

CC- Data collection

SY- Writing

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