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Comparison of Six Automated *Treponema*-Specific Antibody Assays

Borae G. Park,[a] Jihoon G. Yoon,[a] John Hoon Rim,[a] Anna Lee,[b] Hyon-Suk Kim[a]

Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea[a]; Seoul Clinical Laboratory, Seoul Medical Science Institute, Yongin, South Korea[b]

Six different *Treponema* (TP)-specific immunoassays were compared to the fluorescent treponemal antibody absorption (FTA-ABS) test. A total of 615 samples were tested. The overall percent agreement, analytical sensitivity, and analytical specificity of each assay compared to the FTA-ABS test were as follows: Architect Syphilis TP, 99.2%, 96.8%, and 100%; Cobas Syphilis, 99.8%, 99.4%, and 100%; ADVIA Centaur Syphilis, 99.8%, 99.4%, and 100%; HISCL Anti-TP assay kit, 99.7%, 98.7%, and 100%; Immunoticles Auto3 TP, 99.0%, 97.5%, and 99.6%; Mediace TPLA, 98.0%, 98.1%, and 98.0%. All results that were discrepant between the TP-specific assays were associated with samples from noninfectious cases (11 immunoassay false positives and 7 from previous syphilis cases). Our study demonstrated that TP-specific immunoassays generally showed high sensitivities, specificities, and percentages of agreement compared to FTA-ABS, with rare cases of false-positive or false-negative results. Therefore, most TP-specific immunoassays are acceptable for use in screening for syphilis. However, it is important to perform a thorough review of a patient's clinical and treatment history for interpreting the results of syphilis serology.

Syphilis is commonly diagnosed on the basis of the results of a combination of serological tests to detect *Treponema* (TP) antibodies and non-TP antibodies (1). A traditional screening algorithm for syphilis that began with a non-TP assay failed to detect 3% of syphilis cases, in a previous study (2). Recently, a reverse-screening algorithm with an automated TP-specific assay has been recommended by the European Centers for Disease Control and Prevention (ECDC) (3). CDC continues to recommend the traditional algorithm and yet also recognizes the recent trend of the widespread use of the reverse algorithm and recommends extra TP tests to resolve discordant results (4). The reverse algorithm has been found to show superior diagnostic performance, with sensitivities ranging from 99.38% to 99.85%, specificities from 99.98% to 100%, and accuracies from 99.93% to 99.96% compared with a 24.2% missed-diagnosis rate and 75.81% sensitivity of the traditional algorithm (5).

Various automated TP-specific immunoassays have been developed that use either whole cells or antigens, such as 15TpN, 17TpN, and 47TpN, derived from the Nichols strain of *Treponema pallidum*, to detect IgG, IgM, or total immunoglobulins (1). Initially, enzyme immunoassays (EIAs) were commonly used to detect TP-specific antibodies (5, 6). However, the use of chemiluminescence immunoassays (CLIAs) to detect TP-specific IgG and IgM antibodies has been gradually increasing (6, 7). Additionally, quantitative TP-specific immunoassays using turbidimetry, based on a latex agglutination method, have been widely used in Asia. However, comparative analyses of the performances of these various methods are lacking.

The aim of this study was to evaluate the performances of 6 commonly used TP-specific immunoassays, including CLIAs and turbidimetry assays, in comparison with the performance of the fluorescent treponemal antibody absorption (FTA-ABS) test.

MATERIALS AND METHODS

Study design. A total of 615 samples were tested using the 6 kinds of automated TP-specific immunoassays. These samples included 613 left-over serum samples that had been sent for TP or non-TP assay and 2 international standards for syphilis (no. 05/122 and 50/132; National Institute for Biological Standards and Control [NIBSC], Hertfordshire, United Kingdom). The samples included those from 105 medical check-ups of healthy individuals, 179 preoperative evaluations of patients with variable underlying disease, and 329 suspected cases of current or previous syphilis. The median age of all patients was 48 years (range, 5 to 93 years). All immunoassay results were compared with those of the FTA-ABS test (Zeus Scientific, NJ, USA). In addition, the Venereal Disease Research Laboratory (VDRL) test was performed and clinical history was reviewed for samples with discordant TP-specific assay results. Samples were stored at 4°C until all testing was complete. The study protocol was reviewed by the institutional review board at our hospital.

***Treponema*-specific immunoassays.** All six kinds of TP-specific immunoassays were performed following the manufacturer's instructions. Assays evaluated included Architect Syphilis TP (Abbott Diagnostics, Tokyo, Japan), Cobas Syphilis (Roche Diagnostics, Mannheim, Germany), ADVIA Centaur Syphilis (Siemens Healthcare Diagnostics, NY, USA), HISCL Anti-TP assay kit (Sysmex Corporation, Kobe, Japan), Immunoticles Auto3 TP (A & T Corporation, Kanagawa, Japan), and Mediace TPLA (Sekisui Medical Co, Tokyo, Japan). Two of these assays are quantitative assays: Immunoticles Auto3 TP and Mediace TPLA. Characteristics of the 6 different immunoassays are presented in Table 1.

FTA-ABS and VDRL tests. The FTA-ABS assay, which uses the nonviable Nichols strain of *T. pallidum* for detection of TP-specific total antibodies, was performed according to the manufacturer's instructions. Every batch of patient samples was tested with negative and positive controls, and the results of positive samples were graded on a scale from +1 to +4.

The quantitative VDRL test was performed using a BD VDRL Antigen kit (Becton, Dickinson and Company, MD, USA) according to the manufacturer's instructions. Serum samples were quantitated to an endpoint titer of 1:32.

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Address correspondence to Hyon-Suk Kim, kimhs54@yuhs.ac.

TABLE 1 Characteristics of six different immunoassays to detect *Treponema*-specific antibody

Characteristic	Description[a]					
	Abbott	Roche	Siemens	Sysmex	A & T Co.	Sekisui
Reagent	Syphilis TP	Cobas Syphilis Total antibodies to *Treponema pallidum*	Syphilis (SYPH)	HISCL Anti-TP assay kit	IMMUNOTICLES Auto3 TP	Mediace TPLA
Instrument	Architect 2000i (Abbott)	Cobas e601 (Roche)	ADVIA Centaur (Siemens)	HISCL-2000i (Sysmex)	CA-400 (Furuno, Hyogo, Japan)	CA-400
Principle	Chemiluminescent microparticle immunoassay	Electrochemiluminescence immunoassay	Direct chemiluminometric immunoassay	Chemiluminescent enzyme immunoassay	Immunoturbidimetry	Immunoturbidimetry
Quantitative or qualitative	Qualitative	Qualitative	Qualitative	Qualitative	Quantitative	Quantitative
Target antigen[b] (TpN15, TpN17, and TpN47)	Microparticles coated with recombinant *Treponema*-specific antigens; acridinium-labeled anti human IgG and IgM conjugate	Ruthenium-labeled biotinylated *Treponema*-specific antigen; streptavidin-coated microparticle	Acridinium ester-labeled biotinylated *T. pallidum* recombinant antigens (TpN15 and TpN17); streptavidin-coated magnetic latex particle	Alkaline phosphatase-labeled biotinylated recombinant *Treponema*-specific antigens; streptavidin-coated magnetic particles	Nichols strain of *T. pallidum*; *T. pallidum*-sensitized polystyrene latex particle	*Treponema*-specific antigen; *Treponema*-specific antigen-sensitized latex
Calibrator type (no. of points)	1	2	2	1	6	5
Specimen(s)	Plasma, serum	Plasma, serum	Plasma, serum	Plasma, serum	Plasma, serum	Serum
Sample vol (μl)	30	10	100	20	17	16
S/CO or COI[c]	S/CO, 1.0	COI, 1.0	Index, 1.0	COI, 1.0	10 U[d]	10 TU[e] (1 TU = 2 mIU)
Total assay time (min)	29	18	29	17	11	11
Linear measurable analytical range					3–300 U	5–250 TU

[a] All assays included one positive control and one negative control.
[b] All target antigens are composed of TpN15, TpN17, and TpN47, except for Siemens Syphilis.
[c] S/CO, signal/cutoff ratio; COI, cutoff index.
[d] U, unit.
[e] TU, titer unit.

All FTA-ABS and VDRL tests were reviewed by 2 clinical pathologists in the laboratory.

Neutralization assay using TP-specific antigen. Neutralization assay reagents were additionally provided for two quantitative immunoturbidimetric assays: Immunoticles Auto3 TP and Mediace TPLA. Purified TP antigens provided by each vendor were mixed with patient samples and incubated for 30 min at room temperature. Raw samples and neutralized samples were tested at the same time. If the TP antibody titer was considerably lower than the value determined before neutralization, the sample was considered a true positive for TP-specific antibodies.

Statistical analysis. Statistical analyses were performed using MedCalc Statistical Software version 15.6.1 (MedCalc Software bvba, Ostend, Belgium). We evaluated 6 TP assays for analytical sensitivity and specificity and for percent agreement by kappa (κ) coefficients. Linear regression analysis was used to compare quantitative results, and the Kruskal-Wallis test was used to compare results among different groups. The Mann-Whitney *U* test was used to evaluate differences between 2 groups. *P* values of <0.01 were considered statistically significant.

RESULTS

Results of automated immunoassays. A total of 157 samples from 155 patients (median age, 56 years; range, 19 to 93 years) and 2 standards showed positive results. The overall percentages of agreement and corresponding κ values of each assay's results compared with those of FTA-ABS were as follows: for Architect Syphilis TP, 99.2%, $\kappa = 0.978$; for Cobas Syphilis, 99.8%, $\kappa = 0.996$; for ADVIA Centaur Syphilis, 99.8%, $\kappa = 0.996$; for the HISCL Anti-TP assay kit, 99.7%, $\kappa = 0.991$; for Immunoticles Auto3 TP, 99.0%, $\kappa = 0.974$; and for Mediace TPLA, 98.0%, $\kappa = 0.949$ (Table 2). The analytical sensitivities of the assays were 96.8%, 99.4%, 99.4%, 98.7%, 97.5%, and 98.1%, and the specificities were 100%, 100%, 100%, 100%, 99.6%, and 98.0%, respectively. Eighteen samples that showed results that were discrepant between the TP-specific assays were from noninfectious cases. Seven of these results were from cases confirmed by clinical history to represent previous syphilis cases, and 11 of these results (2 from healthy individuals, 1 from a 90-year-old allergic dermatitis patient without a history of syphilis, and 8 from patients with various malignancies who were tested to evaluate preoperative condition without infectious symptoms) were considered to represent false-positive reactions of the immunoassay (Table 3).

Neutralization assay using TP-specific antigen in a quantitative turbidimetry assay. The 11 false-positive, nonspecific reactions were found only in the turbidimetry immunoassays, i.e., Immunoticles Auto3 TP and the Mediace TPLA assay (Table 3). Nine of these results were from the Mediace TPLA. All 11 results were confirmed to be false positives by neutralization assay. Ten true-positive sera, diluted to various concentrations, were tested in parallel using the neutralization assay as a control; none of the results from these samples were identified as false positives.

Analyses of the quantitative assays. The linear analytic measurable range (AMR) for the Immunoticles Auto3 TP assay was 0 to 300 U/ml and for the Mediace TPLA was 0 to 250 U/ml, according to the manufacturer's instructions. A total of 531 data points were within the linear AMR. The 2 quantitative assays showed good correlation by linear regression analysis within the linear AMR ($y = 1.3674\times + 1.7779$, $R^2 = 0.8805$, $P < 0.0001$) (Fig. 1). All data points over the AMR were obtained after dilution, and 9 outliers were excluded. Outliers were defined as those points that were over 1,000 units in Immunoticles Auto3 TP but less than 250 titer units (TU) in Mediace TPLA. The regression analysis of these

TABLE 2 Evaluation of various *Treponema* assays in comparison with the FTA-ABS[a] assay

Assay and result	No. of FTA-ABS test results		% sensitivity	% specificity	% agreement	Kappa value (range) [SE]
	Reactive	Nonreactive				
Abbott Architect						
Reactive	152	0	96.8	100.0	99.2	0.978 (0.960–0.997) [0.010]
Nonreactive	5	458				
Roche Cobas						
Reactive	156	0	99.4	100.0	99.8	0.996 (0.987–1.000) [0.004]
Nonreactive	1	458				
ADVIA Centaur						
Reactive	156	0	99.4	100.0	99.8	0.996 (0.987–1.000) [0.004]
Nonreactive	1	458				
Sysmex HISCL						
Reactive	155	0	98.7	100.0	99.7	0.991 (0.980–1.000) [0.006]
Nonreactive	2	458				
A&T Immunoticles						
Reactive	153	2	97.5	99.6	99.0	0.974 (0.954–0.998) [0.010]
Nonreactive	4	456				
Sekisui Mediace						
Reactive	154	9	98.1	98.0	98.0	0.949 (0.921–0.978) [0.014]
Nonreactive	3	449				

[a] FTA-ABS, fluorescent treponemal antibody absorption.

606 data points showed better correlation ($y = 1.4243\times + 15.6419$, $R^2 = 0.9679$, $P < 0.0001$) than the analysis with the full range of data (Fig. 1).

In addition, quantitative results from immunoassays were associated with graded results of the FTA-ABS assay (Fig. 2). Quantitative assays showed false-positive results, but these assays could differentiate between high-grade positivity (score of +3 or +4) and low-grade positivity (+1 or +2). However, there were no statistically significant differences between results with scores of +3 and +4 or between those with scores of +1 and +2 in the Immunoticles Auto3 TP and Mediace TPLA assays (Fig. 2). The units of the 2 biological standards were 0.3 IU/ampoule (for standard 05/122) and 3 IU/ampoule (for standard 05/132). They measured 455 and 3,400 TU by Mediace TPLA and 260 and 1,610 units by Immunoticles Auto3 TP assays.

DISCUSSION

Our findings indicated that commercial TP-specific immunoassays currently in use show high sensitivities, specificities, and percentages of agreement compared with FTA-ABS. However, rare cases of false positives or false negatives resulted. False-positive reactions in both non-TP-specific and TP-specific assays are more likely to be seen under specific conditions, such as viral or bacterial infection, autoimmune disease, pregnancy, postimmunization status, diabetes, and old age (1, 6). Therefore, without a known history of syphilis or a positive anti-TP IgM test result, persistent

TABLE 3 Data on 18 results that were discordant between *Treponema*-specific immunoassays of 615 specimens

Result type(n)[a]	Total no. of results	Test reactivity[b]							
		VDRL (titer)	FTA-ABS (score)	Abbott	Roche	Siemens	Sysmex	A&T Co.	Sekisui
False positive (11)	7								R
	2							R	
	2	R (1:1)							R
False negative (7)	1		R (+1)						
	1		R (+3)	R	R	R	R	R	
	1		R (+2)		R	R			R
	2	R (1:1)	R (+2)		R	R	R	R	R
	1	R (1:1)	R (+2)	R	R	R	R		R
	1	R (1:1)	R (+1)		R	R	R		

[a] False-positive or false-negative results in any of the *Treponema*-specific immunoassays were confirmed by neutralization assay and clinical history of patients.
[b] VDRL, Venereal Disease Research Laboratory; FTA-ABS, fluorescent treponemal antibody absorption; R, reactive.

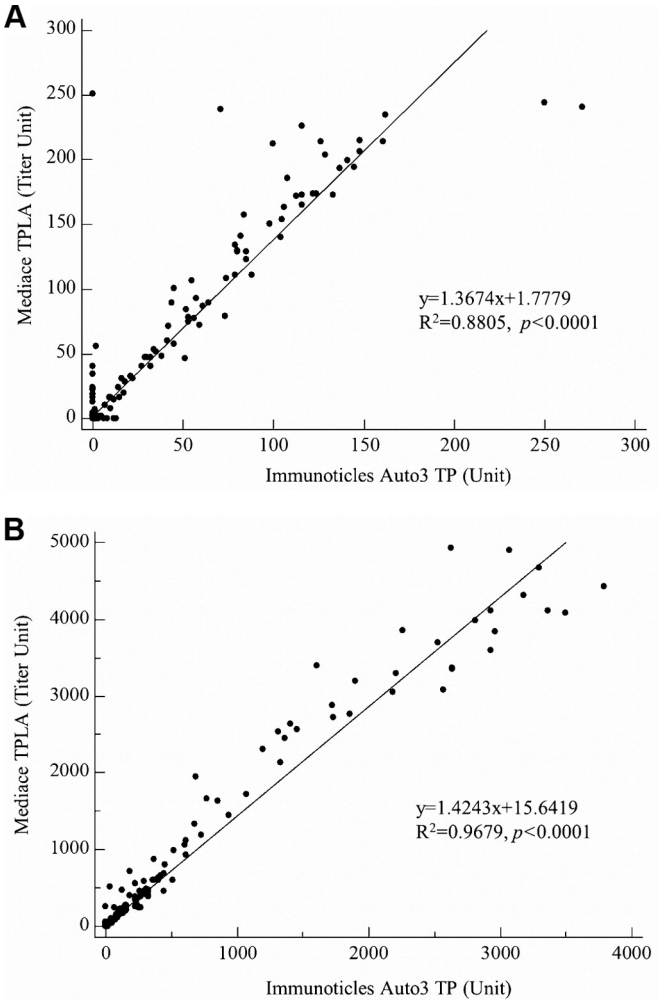

FIG 1 Linear regression of two quantitative *Treponema*-specific immunoassays. (A) A total of 531 data points within the linear analytical measurable range (AMR) of each assay (300 units for Immunoticles and 250 titer units for Mediace). (B) A total of 606 data points obtained after dilution of samples, showing the data over the AMR and excluding 9 data points as outliers.

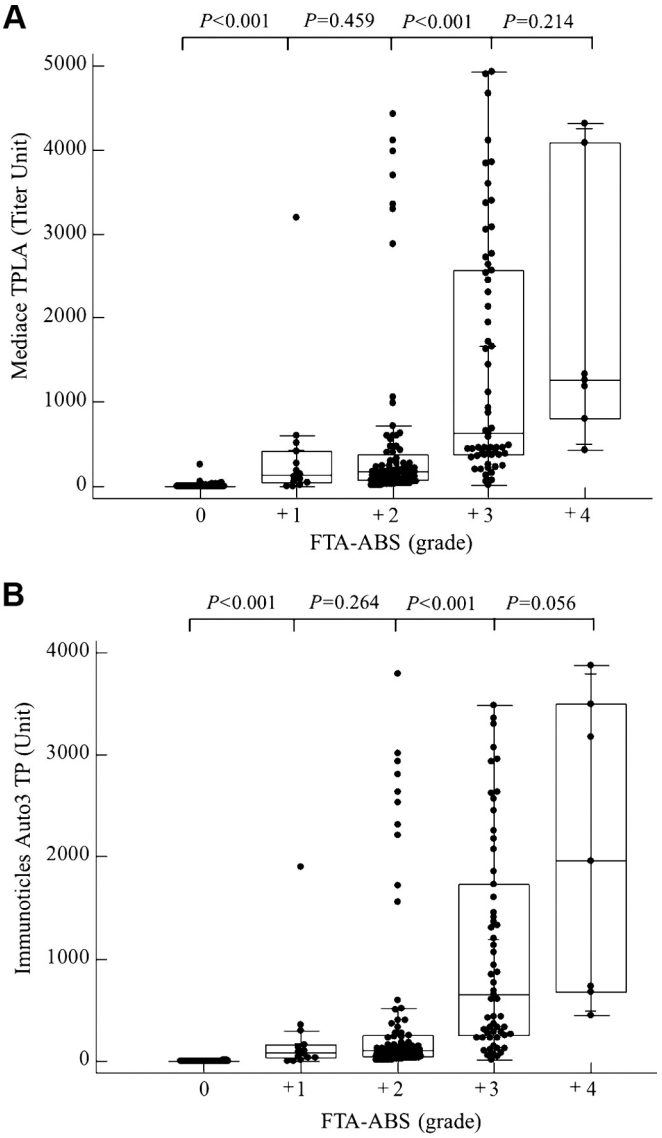

FIG 2 Comparison between the quantitative results of *Treponema*-specific immunoassays and grades of the FTA-ABS (fluorescent treponemal antibody absorption) test. (A) Comparison between Mediace TPLA and FTA-ABS results. (B) Comparison between Immunoticles Auto3 TP and FTA-ABS results. All *P* values were determined by Mann-Whitney *U* tests.

or transient reactivity in a non-TP or TP assay should be considered a false-positive reaction. In our study, false-positive results were seen only with turbidimetric immunoassays, especially Mediace TPLA. Previously, 1.2% false positives were reported for a latex-agglutinated assay used on samples from non-syphilis patients (8). The neutralization assay was employed to rule out false-positive reactions, and it effectively distinguished between true and false-positive reactions. Therefore, neutralization assay confirmation should be considered, especially with results of Mediace TPLA testing.

Additionally, false-positive results can be ruled out by algorithm. If positive TP results with negative non-TP results are observed in a patient without a history of treatment, a second TP-specific test can be performed to rule out early or late/latent disease (4, 9).

False-negative non-TP tests can result from the prozone phenomenon in early, secondary, or latent syphilis. This phenomenon has rarely been observed to affect TP-specific assays (1, 6). In addition, insufficient antibody production, usually in the 2 to 4 weeks after infection, might lead to false-negative results in TP-specific immunoassays. False-positive results are rare in active syphilis cases because most immunoassays currently in use can detect both the IgM and IgG subtypes of TP-specific antibodies. According to our data, all 7 false-negative results were from previous or treated syphilis cases.

In this study, results that were discordant between the various TP-specific immunoassays were observed. Regardless of which algorithm is used and because of the analytical limitations of syphilis immunoassays, it is important for the clinician always to take the clinical history of a patient into consideration.

Several commercial, quantitative TP assays have been introduced, and our study showed good correlation between the 2 quantitative turbidimetric assays. In addition, quantitative results

correlated with low and high titer results from FTA-ABS testing. However, the quantitative results from testing of the biological standards from NIBSC (provided in 2014) did not match to the unit value of each material in our study. In our study, Mediace TPLA and Immunoticles Auto3 TP measured NIBSC standard material values of 0.3 IU for *Treponema*-specific IgG and IgM and measured 3 IU of syphilis antibody as 455 TU and 260 units and as 3,400 TU and1,610 units, respectively. Thus, the unit values of the TPLA results for standards should be reevaluated.

Previous reports have shown that quantitative results of syphilis assays differed according to the clinical cases and that TP antibody values over 1,000 U were helpful to determine syphilis infection (8). However, the linear AMR values of quantitative assays are under 300 U. Therefore, sufficient dilutions may be necessary in quantitative assays to differentiate infectious from non-infectious (previous) syphilis cases.

Although hands-on times did not differ between assays, the analytical assay times were shorter in quantitative turbidimetric assays than in qualitative immunoassays (Table 1). Additionally, the costs of turbidimetric assays ($1 to $2) were lower than those of immunoassays ($3 to $5). However, the quantitative turbidimetric assays required a longer analytical time for positive samples and were relatively expensive in areas with a high prevalence of syphilis due to the need to include a dilution process of samples to show which data points were above the AMR. Therefore, the prevalence of syphilis must be considered in order to determine the kind of TP-specific assay to be used in laboratory.

In recent years, automated assays to detect TP antibodies and reverse algorithm to detect clinical syphilis have been frequently used (10). However, overtreatment following false-positive test results is known to occur. On the other hand, the sensitive detection of TP-specific antibody provides an advantage in areas of low syphilis prevalence (7).

In our study, various TP-specific immunoassays performed well in comparison with the FTA-ABS test. Therefore, these TP-specific immunoassays are sufficient to screen for syphilis. However, it is important to perform a thorough review of each patient's clinical and treatment history in interpreting the results of syphilis serology because of their analytical limitations.

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