

Evaluation of a Fully Automated Treponemal Test and Comparison With Conventional VDRL and FTA-ABS Tests

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Abstract

We evaluated analytic performances of an automated treponemal test and compared this test with the Venereal Disease Research Laboratory test (VDRL) and fluorescent treponemal antibody absorption test (FTA-ABS). Precision performance of the Architect Syphilis TP assay (TP; Abbott Japan, Tokyo, Japan) was assessed, and 150 serum samples were assayed with the TP before and after heat inactivation to estimate the effect of heat inactivation. A total of 616 specimens were tested with the FTA-ABS and TP, and 400 were examined with the VDRL. The TP showed good precision performance with total imprecision of less than a 10% coefficient of variation. An excellent linear relationship between results before and after heat inactivation was observed ($R^2 = 0.9961$). The FTA-ABS and TP agreed well with a κ coefficient of 0.981. The concordance rate between the FTA-ABS and TP was the highest (99.0%), followed by the rates between FTA-ABS and VDRL (85.0%) and between TP and VDRL (83.8%). The automated TP assay may be adequate for screening for syphilis in a large volume of samples and can be an alternative to FTA-ABS.

Syphilis is a disease caused by *Treponema pallidum* infection. An estimated 70,000 sexually transmitted infections with *T pallidum* occur each year in the United States,¹ and, worldwide, approximately 12 million new cases of syphilis occurred in 1997.² Also in Korea, the number of newly infected patients reached about 1,500 in 2009 since the lowest number of 134 was recorded in 2002.³ Because this disease has clinical manifestations similar to those of nontreponemal disease, laboratory diagnostic tests with history taking and physical examination are mandatory for the diagnosis. Although there is no single definitive test, identification of *T pallidum* by dark-field microscopy or direct fluorescent antibody staining is considered the “gold standard” for the diagnosis. However, such methods require specimens obtained from moist lesions and need to be performed by an experienced laboratory technician.^{4,5} Polymerase chain reaction–based tests are fairly expensive compared with conventional laboratory tests,^{6–8} and real-time polymerase chain reaction provides limited sensitivities for secondary and latent syphilis.^{9,10} Thus, serologic tests are still widely used for syphilis diagnosis owing to reasonable costs and familiarity to practicing clinicians.

Serologic tests for the diagnosis of syphilis fall into 2 categories: nontreponemal tests and treponemal tests. Nontreponemal tests, which include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin tests, identify both IgG and IgM antilipoidal antibodies produced by the host in response to lipoidal materials released from damaged host cells and lipid from the cell surface of the *T pallidum* itself,^{4,5} whereas treponemal tests detect antibodies specific to *T pallidum* and include the fluorescent treponemal antibody absorption (FTA-ABS), *T pallidum* hemagglutination, and *T pallidum* particle agglutination.

In some countries, including the United States and Korea, nontreponemal tests are used as a first-line test in screening for syphilis, and positive results of nontreponemal tests are confirmed by a treponemal test.⁴ However, this strategy may not properly diagnose cases of late latent syphilis and could delay the diagnosis while confirming with treponemal tests. In these respects, some European countries, such as the United Kingdom and Germany, adopted treponemal tests as a screening test for syphilis.¹¹ In addition, sensitivities of treponemal tests such as the FTA-ABS are known to be higher than those of nontreponemal tests.⁵ Hence, treponemal tests can be more useful in screening for syphilis.^{12,13} However, these conventional tests still require well-trained laboratory technicians to perform manual procedures such as heat inactivation and to interpret the results. For example, antigens need to be freshly prepared for everyday VDRL tests. Biologic false-positive results, especially in nontreponemal tests, are also problematic.^{12,14}

Presently, some immunoassay reagents and autoanalyzers for the detection of antilipoidal antibody or *T pallidum*-specific antibody (TP-specific Ab) are commercially available.^{15,16} Earlier reagents for TP-specific Ab mainly used turbidimetric methods, such as *T pallidum* particle agglutination, and then an automated chemiluminescent microparticle immunoassay was developed. In this study, we evaluated analytic performance of this assay and compared the results with those of other conventional tests for syphilis.

Materials and Methods

Architect Syphilis TP Assay

The Architect Syphilis TP (TP) assay (Abbott Japan, Tokyo, Japan) is a 2-step chemiluminescent microparticle immunoassay for the qualitative detection of antibody to *T pallidum* in serum or plasma using an Architect *i* System (Abbott Laboratories, Abbott Park, IL). During the assay, microparticles coated with recombinant TP antigens (TpN15, TpN17, and TpN47) bind to anti-TP antibodies presented by the specimen. After washing the specimen, mouse antihuman IgG-IgM conjugate labeled with a chemiluminescent molecule of acridinium is added, and the resultant chemiluminescent reaction is measured in relative light units (RLUs), which are directly related to the amount of TP-specific Ab in the specimen. The Architect analyzer calculates a ratio between the sample RLU and cutoff RLU, and samples with a ratio of more than 1.0 were considered positive.

VDRL Test

VDRL was tested using BD VDRL Antigen kits (Becton Dickinson, Sparks, MD) according to the manufacturer's instructions. An antigen suspension was prepared before testing and used within 4 hours. All serum specimens were heated at 56°C for 30 minutes before testing. When an undiluted sample

was reactive for VDRL, semiquantitative tests were performed with a serial dilution of the sample to a 1:32 ratio. The results were interpreted using a microscope at ×100 magnification.

FTA-ABS Indirect Fluorescent Antibody Test

FTA-ABS tests were performed after samples were heated at 56°C for 30 minutes. Commercially available glass slides (Zeus Scientific, Raritan, NJ) on which *T pallidum* substrate had been fixed were used according to the manufacturer's instructions. Every testing batch of patient samples was tested with negative and positive controls with 1+ to 4+ reactivity. The slides were examined with a fluorescence microscope, and positive results were graded on a scale of 1+ to 4+ or recorded as minimally reactive. All minimally reactive samples and those graded as 1+ were repeatedly tested.

Specimens

A total of 616 serum samples, which had been obtained for requested FTA-ABS tests by various departments including general medicine and dermatology, were collected from people who were suspected of having syphilis or who took a medical examination at Severance Hospital of Yonsei University, Seoul, Korea, or Seoul National University Hospital, Seoul, Korea. The mean ± SD age of subjects was 48.8 ± 16.1 years, and there were 313 males and 303 females, including 2 pregnant women. All specimens were stored at -70°C after FTA-ABS tests were finished and were tested with TP. The VDRL was additionally done in 400 specimens, which were obtained from 203 males and 197 females. The medical records were reviewed if the results of the 3 tests did not agree. In addition, 108 serum samples with positive antinuclear antibodies and nonreactive with the VDRL and FTA-ABS tests were assayed on the TP to evaluate possible interference on the TP by antinuclear antibodies. This study was approved by the institutional review board of Severance Hospital.

Precision Performance of the Syphilis TP Assay

Precision performance of the TP assay was assessed based on the guidelines in Clinical and Laboratory Standards Institute document EP15-A.¹⁷ The assay was performed in duplicate with positive and negative control samples and pooled sera with 2 levels twice a day for 5 days, and the resulting RLUs were analyzed.

Effect of Heat Inactivation on the TP Assay

A total of 150 serum samples were assayed on the TP assay twice before and after heat inactivation, and the resultant RLUs were compared.

Statistical Analysis

All statistical analyses were performed by using the Analyse-it Method Evaluation edition software, version 2.22

(Analyse-it Software, Leeds, England). A paired *t* test and linear regression analysis were carried out to evaluate the effect of heat inactivation on the TP assay. The κ coefficients were calculated to estimate the agreement of the results for the VDRL, FTA-ABS, and TP assays. Multiple pairwise comparison of the TP RLUs according to the groups classified by the results of VDRL or FTA-ABS tests were conducted using the Kruskal-Wallis test with Bonferroni correction to compensate for an α statistical error. *P* values less than .05 were considered statistically significant.

Results

Precision of the TP Assay

The precision performance of the TP assay for samples with 4 concentrations (positive control, negative control, pooled serum 1, and pooled serum 2) are summarized in **Table 1**. The coefficient of variation (CV) for within-run

imprecision ranged from 2.0% to 5.9%, and the CV for total imprecision was between 2.4% and 8.2%.

Effect of Heat Inactivation on the TP Assay

The mean \pm SD RLUs for the TP assay were 8.37 ± 10.33 before heat inactivation and 8.19 ± 9.96 after heat inactivation ($n = 150$), and there were small differences between the RLUs (mean \pm SD difference, 0.18 ± 0.74 ; $P = .0033$). On the other hand, the results were well correlated with each other in linear regression analysis ($R^2 = 0.9961$; $P < .0001$), and the following equation was established: $y = 0.9618x + 0.1399$, where *x* represents RLUs before heat inactivation and *y* represents RLUs after heat inactivation.

Comparison Among VDRL, FTA-ABS, and TP Assays

The FTA-ABS and TP assays agreed better, with a κ of 0.981, than the VDRL and FTA-ABS tests ($\kappa = 0.700$) and the VDRL and TP assays ($\kappa = 0.675$) **Table 2** and **Table 3**. The

Table 1
Precision Performance of the Architect TP Assay

Sample	Mean RLUs	Coefficient of Variation (%)			
		Repeatability	Between-Run	Between-Day	Total
Positive control	2.36	5.90	0.00	4.50	7.40
Negative control	0.22	4.80	6.40	2.00	8.20
Pooled serum 1	25.31	2.00	1.40	0.00	2.40
Pooled serum 2	0.89	3.20	2.50	4.00	5.70

RLUs, relative light units.

Table 2
Comparison of VDRL, FTA-ABS, and Architect TP Results

Comparative Test	VDRL			Concordance Rate (%)	κ (95% CI)	<i>P</i>
	Reactive	Nonreactive	Total			
FTA-ABS						
Reactive	155	15	170			
Nonreactive	45	185	230			
Total	200	200	400	85.0	0.700 (0.631-0.769)	<.0001
Architect TP						
Positive	152	17	169			
Negative	48	183	231			
Total	200	200	400	83.8	0.675 (0.604-0.746)	<.0001

CI, confidence interval; FTA-ABS, fluorescent treponemal antibody absorption test; VDRL, Venereal Disease Research Laboratory test.

Table 3
Comparison of the Results Between the FTA-ABS and Architect TP

Architect TP	FTA-ABS			Concordance Rate (%)	κ (95% CI)	<i>P</i>
	Reactive	Nonreactive	Total			
Positive	306	3	309			
Negative	3	304	307			
Total	309	307	616	99.0	0.981 (0.965-0.996)	<.0001

CI, confidence interval; FTA-ABS, fluorescent treponemal antibody absorption test.

concordance rate between the results of the FTA-ABS and VDRL tests was 85.0%, and between the TP and VDRL tests, it was 83.8%. The results between the TP and FTA-ABS tests showed the highest concordance rate, 99.0%.

The RLUs for the TP assay were significantly lower in VDRL-nonreactive specimens than in all other VDRL-reactive groups ($P < .0001$) (Figure 1A). There were also significant differences in the TP RLUs between the VDRL 1:1 weakly reactive and 1:2 reactive groups ($P = .0031$), between the VDRL 1:1 weakly reactive and more than 1:8 reactive groups ($P = .0011$), and between the VDRL 1:1 reactive and more than 1:8 reactive groups ($P = .0083$). Otherwise, there was no difference in RLUs according to VDRL reactivity. The median TP RLU was 0.19 for the VDRL-nonreactive group ($n = 200$), 7.49 for the 1:1 weakly reactive group ($n = 107$), 7.92 for the 1:1 reactive group ($n = 58$), 21.67 for the 1:2 reactive group ($n = 22$), 20.89 for the 1:4 reactive group ($n = 6$), and 28.02 for the group designated more than 1:8 reactive ($n = 7$).

The RLUs of the TP assay were significantly different between most of the groups divided by FTA-ABS reactivity (overall, $P < .0001$) except between the FTA-ABS 1+ and 2+ ($P = .5655$) and between the FTA-ABS 3+ and 4+ groups ($P = .7853$) (Figure 1B). The median TP RLUs for each FTA-ABS

reactivity group were as follows: 0.20 in the nonreactive group ($n = 252$), 4.11 in the minimally reactive group ($n = 34$), 9.43 in the 1+ reactive group ($n = 42$), 10.87 in the 2+ reactive group ($n = 139$), 18.56 in the 3+ reactive group ($n = 42$), and 28.28 in the 4+ reactive group ($n = 7$).

Specimens With Antinuclear Antibodies as Assay-Interfering Substances

A total of 108 samples with various intensities of antinuclear antibody from 1:40 to higher than 1:160 were tested in the TP assay. All specimens were negative for TP.

Discussion

In this study, we evaluated the analytic performance of an automated treponemal test, the Architect TP assay, and compared the results with those of other conventional tests. The TP assay showed good precision performance with a total imprecision of less than 10% CV and high agreement with conventional tests for syphilis, especially almost concordant results with the FTA-ABS. The correlation between the TP and VDRL tests was slightly low, which is similar to previous reports.^{6,12,15,18}

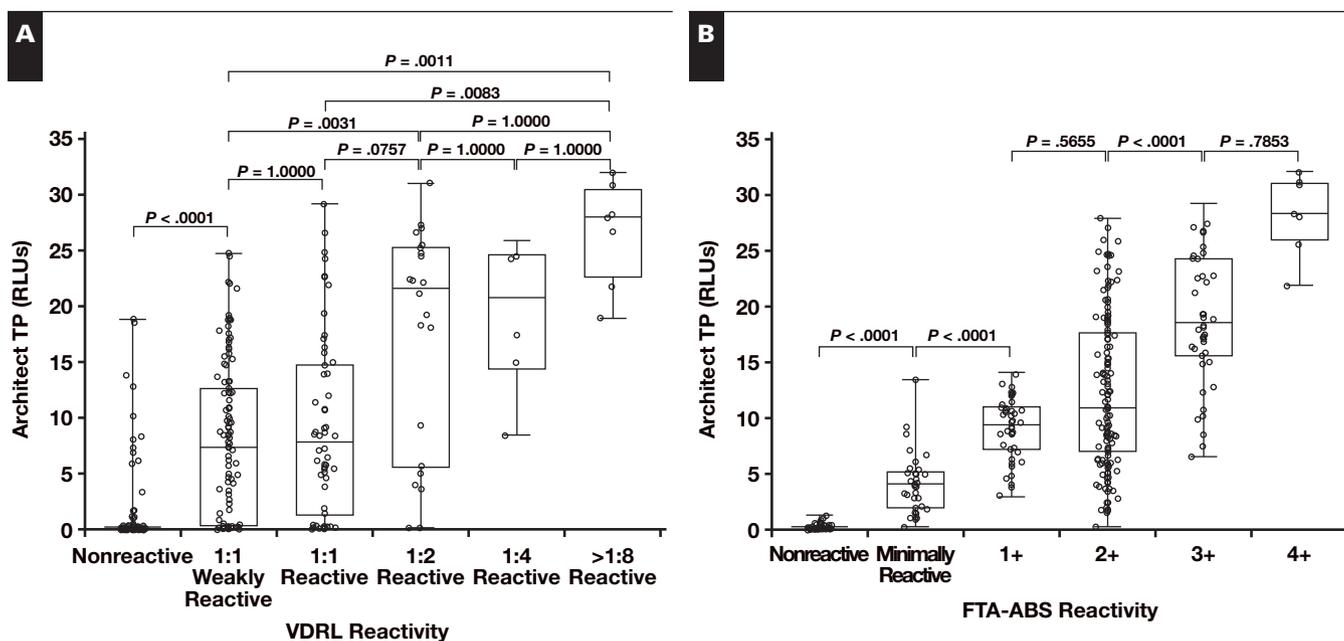


Figure 1 Architect TP relative light units (RLUs) according to reactivity of the fluorescent treponemal antibody absorption (FTA-ABS) and Venereal Disease Research Laboratory (VDRL) tests. **A**, The RLUs were lower in the VDRL nonreactive group than in all other VDRL reactive groups. There were also significant differences in TP RLUs between VDRL 1:1 weakly reactive and 1:2 reactive groups, between VDRL 1:1 weakly reactive and more than 1:8 reactive groups, and between VDRL 1:1 reactive and more than 1:8 reactive groups. **B**, The RLUs of the TP were different between most of the groups by FTA-ABS reactivity except between the FTA-ABS 1+ and 2+ and between the FTA-ABS 3+ and 4+ groups. Upper and lower ends of boxes indicate upper and lower quartiles, respectively; inner lines, median values; whiskers, maximum and minimum values; and circles, individual values.

In addition, we evaluated effects of the heat inactivation process on TP assay results to identify whether heat-inactivated samples can be reliably tested on the TP assay. Because the FTA-ABS and VDRL tests are performed after heat inactivation, it is convenient to confirm the results of the VDRL and FTA-ABS tests with the TP assay using the samples after the former 2 tests are finished. As a result, the TP RLUs were slightly lower in heat-inactivated samples, but there was a linear relationship between the results obtained before and after heat inactivation, and the difference between the results was negligible. Therefore, heat-inactivated samples would be reliably assayed by the TP, although this is not recommended by the manufacturer.

In our data, there were some discrepancies in the VDRL, FTA-ABS, and TP results, and most discrepancies seemed to be caused by false-positive results in the VDRL test. A total of 45 specimens with VDRL-reactive and FTA-ABS–nonreactive results might have been caused by false positivity in the VDRL, which was assumed after reviewing patients' records. There were 48 samples that were reactive with the VDRL and negative with the TP assay, and 45 of them were the ones that were positive with the VDRL and negative with the FTA-ABS test. In the other 3 samples, the VDRL test results were thought to be false-positive because the patients did not have any evidence of syphilis infection. The mean TP RLU value for 48 VDRL-reactive and TP assay–negative specimens was 0.27 (95% confidence interval, 0.06–0.93). The reasons for false positivity in the VDRL results corresponded with well-known causes such as systemic lupus erythematosus, malignancies, and hepatitis. Of the 2 pregnant women, 1 also had a false-positive result on the VDRL.

Meanwhile, 15 VDRL-nonreactive and FTA-ABS–reactive specimens were obtained from patients already treated or with late latent syphilis, including the other pregnant woman. Additional 2 specimens with nonreactive VDRL and positive TP assay results might have resulted from the false-positive TP assay. The RLUs of the TP assay in these 2 cases were slightly higher than the cutoff (1.21 and 1.07). In addition, 3 FTA-ABS–nonreactive and TP assay–positive specimens were supposed to be false-positive by TP, whereas another 3 specimens with FTA-ABS–reactive and TP-negative results were presumed to be false-positive in the FTA-ABS test. Among the latter 3 cases, 2 samples were minimally reactive in the FTA-ABS test, and the patients had systemic lupus erythematosus or hepatitis. Another patient had FTA-ABS reactivity of 2+, but there was no clinical reason for the reactivity.

The VDRL test is useful for monitoring syphilis disease activity and treatment response.^{4,5,8,14} However, the sensitivity of the VDRL test is lower than that of treponemal tests such as the FTA-ABS, especially in primary and late latent syphilis.^{4,5,14} In our study, all VDRL-nonreactive and FTA-ABS– or TP assay–positive samples were also obtained from

already treated patients or patients with late latent syphilis. Assuming the diagnoses in patients' medical records are correct, the sensitivity of the VDRL was 76%, which is similar to that in other studies.^{5,6,14,15,19} In this respect, there are possibilities for a wrong or delayed diagnosis when the VDRL test is used to screen for syphilis. Therefore, nontreponemal tests are appropriate for monitoring, and treponemal tests are suitable for screening and diagnosing syphilis. However, the procedure for FTA-ABS is complicated, and results may vary according to the person who interprets the fluorescent microscopic images. In this sense, the fully automated Architect TP assay, which does not involve complicated sample preparation process, has an advantage over the FTA-ABS test. In our study, TP assay results were highly concordant with FTA-ABS results ($\kappa = 0.981$). Some countries adopt different strategies for screening and confirming syphilis infections as stated earlier, and these strategies would be standardized.

In addition, cerebrospinal fluid (CSF) is generally screened by the FTA-ABS test for the diagnosis of neurosyphilis, and the VDRL can be performed as a confirmatory test^{15,20} because the FTA-ABS has high sensitivity and the VDRL has high specificity for the detection of neurosyphilis in CSF samples. We experimentally tested 44 CSF specimens to evaluate the usefulness of the Architect TP for the diagnosis of neurosyphilis. However, results for 30 samples could not be obtained owing to a technical problem. Because the antibody quantity in CSF is usually low, the antigen quantity in the TP reagent kit may need to be optimized before applying it to CSF specimens, and further studies on the usefulness of the TP assay for the diagnosis of neurosyphilis would be necessary.

The Architect TP assay showed good precision and correlation with conventional syphilis tests, especially the FTA-ABS, which is known as the most sensitive treponemal test for syphilis. This automated TP assay is considered adequate for screening for syphilis in a large volume of samples owing to its convenience, and it can be an alternative to FTA-ABS.

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