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Evaluation of a Rapid Immunochromatographic Treponemal Antibody Test Comparing the *Treponema Pallidum* Particle Agglutination Assay

Jong-Han Lee,[1] Chae Seung Lim,[1] Min-Geol Lee,[2] and Hyon-Suk Kim[3]*

[1]*Department of Laboratory Medicine, Korea University College of Medicine, Seoul, Korea*
[2]*Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea*
[3]*Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea*

Background: In addition to conventional tests, several methods for detection of treponema-specific antibodies in clinical settings have been recently introduced. We aim to comparatively evaluate a rapid immunochromatographic test (ICT) for *Treponema pallidum* specific antibody (SD Bioline Syphilis 3.0) and the *T. pallidum* particle agglutination (TPPA) assay. *Methods:* In all, 132 serum samples from 78 syphilis patients and 54 syphilis-negative controls were analyzed. SD Bioline Syphilis 3.0 test (Standard Diagnostic, Inc., Yongin, Korea) was evaluated and compared to Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). All discrepant results between the two assays were repeatedly tested and evaluated by the fluorescent treponemal antibody-absorption (FTA-ABS) assay. Test reproducibility and 95% limit of detection of SD Bioline Syphilis 3.0 were determined across three different lots for seven consecutive days in triplicate. Interference due to autoantibodies and pregnancy was also tested. *Results:* Percent agreement between SD Bioline Syphilis 3.0 and TPPA assays was 99.2%. Sensitivity and specificity were 100%, respectively. In TPPA assay, test-to-test, day-to-day, and lot-to-lot variations were not identified until 1:320 titer (eightfold dilutions). There was no interference due to the presence of antinuclear antibodies or samples or pregnancy. *Conclusions:* Percent agreement of SD Syphilis 3.0 and TPPA was very good. Sensitivity and specificity were appropriate for *T. pallidum* antibody detection. Thus, a rapid ICT could be suitable for syphilis antibody detection. J. Clin. Lab. Anal. 29:383–386, 2015. © 2014 Wiley Periodicals, Inc.

Key words: Syphilis; rapid immunochromatographic test; *Treponema pallidum* particle agglutination (TPPA); agreement

Syphilis is one of the major infectious diseases and was recently found in about 11 million adults among developing countries in 2005 (1). In Korea, the prevalence of syphilis has rapidly decreased since the 1970s, and it was approximately 0.2% in 2000. However, recent studies have suggested a slight increase in the prevalence, which later resulted in a plateau (2). Despite this finding, primary and secondary syphilis in immunocompromised patients have increased worldwide in the last decade (3). Therefore, appropriate screening, diagnosis, and treatment protocols are needed and well established (4–6). A recently highlighted concern is that traditional diagnostic approaches should be replaced by a reverse syphilis testing algorithm. The reverse algorithm is a treponemal-specific test applied as a screening tool followed by a quantitative nontreponemal assay that diagnoses active disease and monitors patients' treatment response (7). Of the various treponemal assays, the *Treponema pallidum* particle agglutination (TPPA) assay is more widely used than the fluorescent treponemal antibody-absorption (FTA-ABS) test for confirmatory testing of syphilis (8). The aim of this study was to evaluate a rapid immunochromatographic test (ICT), SD Bioline Syphilis 3.0, for its clinical usage.

*Correspondence to: Hyon-Suk Kim, Department of Laboratory Medicine, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea. E-mail: kimhs54@yuhs.ac

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A total of 132 serum samples from 78 patients with confirmed syphilis and 54 healthy nonsyphilis control specimens were collected and preserved at −70°C until analyses. Accurate analyses of the patients according to the series of syphilis stage could not be carried out owing to certain characteristics and infrequency of the disease in Korea. A rapid ICT, SD Bioline Syphilis 3.0 (Standard Diagnostic, Inc., Yongin, Korea), was evaluated and compared to the Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). SD Bioline Syphilis 3.0 is a solid-phase ICT used for the detection of antibodies of *T. pallidum*. The kit contains reagents and membrane strips that are precoated with three kinds of recombinant *T. pallidum* antigens, including rTpN15, rTpN17, and rTpN47, on the test band region. Binding of these antigens to the antibodies present in the patient's sample and the secondary colloidal gold conjugate leads to the formation of visible bands. Serodia TPPA assay is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigens. For quantitative results, the antibody titer was determined as the final dilution showing a positive result. A final dilution of 1:80 or more was considered as a positive reaction. Discrepancy between SD Bioline Syphilis 3.0 and TPPA assay results were repeatedly tested by different technologists who simultaneously performed the FTA-ABS assay. The FTA-ABS assay is performed using a commercially available test kit (Zeus Scientific, Raritan, NJ) in which *T. pallidum* substrate is fixed on a glass slide according to the manufacturer's protocol. Percent agreement was calculated as kappa values, which were used to categorize the result as very good (0.81–1.0), good (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), or poor (0–0.2) (9). The sensitivity and specificity of the assay were analyzed by defining true positive cases that were based on the results of TPPA and FTA-ABS assays and the patients' medical chart review. Reproducibility of SD Bioline Syphilis 3.0 test was analyzed according to modified CLSI guidelines EP12-A2 (10). Reproducibility and limit of detection were determined by repeating the tests with two-fold serial dilutions of three kinds of pooled positive serum samples diluted up to 1:128 (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128), reflecting the treponemal antibody concentrations. We performed the tests in triplicate over a period of seven consecutive days using three different lots (lot numbers: 025103A, 025118C, and 025120A). In all, a total of 504 tests were carried out for this evaluation. Fifteen antinuclear antibody (ANA) positive samples and ten samples from pregnant women were additionally tested for production of any false reactions by SD Bioline Syphilis 3.0.

The percent agreement between SD Bioline Syphilis 3.0 and Serodia TPPA was 99.2% (kappa: 0.984, 95% CI: 0.954–1.015). Only one discrepant result was observed among 132 subjects (Table 1). This discrepant sample showed a negative SD Bioline Syphilis 3.0 and positive result (1:320) of Serodia TPPA assays, respectively, and the sample showed minimally reactive with the FTA-ABS test. This sample originated from a 61-year-old male patient who was suspected of herpes simplex virus infection or drug effect rather than syphilis infection based on his dermatologist's review. The sensitivity and specificity of SD Bioline Syphilis 3.0 was 100% (95% CI: 95.3–100), 100% (95% CI: 93.3–100) and Serodia TPPA assay was 100% (95% CI: 95.3–100), 98.2% (95% CI: 90.1–99.7), respectively. The reproducibility among replica specimens, days, and lots showed 100% positivity with the pooled TPPA-positive serum at titer values of 1:2560 (onefold), 1:1280 (twofold), 1:640 (fourfold), and 1:320 (eightfold). However, titer values of 1:160 (16-fold), 1:80 (32-fold), 1:40 (64-fold), 1:20 (128-fold), 1:10 (256-fold), and 1:5 (512-fold) of the pooled serum showed 88.9%, 85.7%, 25.4%, 1.6%, 1.6%, and 0% positivity, respectively. The 95% limit of detection of SD Bioline Syphilis 3.0 was between 1:40 titer (64-fold) and 1:20 titer (128-fold), and 50% limit of detection was between 1:80 titers (32-fold) and 1:40 titers (64-fold) of TPPA, respectively. No false-positive results were obtained due to the presence of ANAs in 15 patients and samples from ten pregnant women.

TABLE 1. Percent Agreement and Kappa Values of Rapid SD Bioline and TPPA Assays

		TPPA		True syphilis infection	
		+	−	+	−
SD Bioline-Syphilis 3.0	+	78	0	78	0
	−	1	53	0	54

Percent agreement: 99.2%.
Kappa value: 0.984.
95% confidence interval: 0.954–1.015.
The strength of agreement is considered to be very good.
TPPA, *Treponema pallidum* particle agglutination.

In secondary and latent stages of syphilis except primary syphilis, the treponemal test is very sensitive and approximately 84% of cases of syphilis remain treponemal test positive for life (8). In clinical settings, the commonly used methods for detection of *T. pallidum* antibodies are *T. pallidum* hemagglutination (TPHA), TPPA, and FTA-ABS assays. TPPA has been reported nearly similar sensitivity as the FTA-ABS assay in primary syphilis cases, and it is as useful as the rapid plasma regain (RPR) test in monitoring therapy (11). It is less subjective than FTA-ABS assay and the results are easier to interpret than the classical microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP) (11). TPPA assay was also reported to be useful in the diagnosis of neurosyphilis

using cerebrospinal fluid (CSF) samples (12). The rapid ICT for syphilis detection has some advantages, such as completion of the test within 30 min without the use of complex equipments and technical skills. We found that the concordance rate of SD Bioline Syphilis 3.0 and Serodia TPPA assays was very good. The overall diagnostic performances of these two assays were the same or even superior to that of the TPPA assay. A previous multicenter evaluation reported that SD Bioline Syphilis 3.0 showed a diagnostic sensitivity of 95% (95% CI: 92.8–97.1) and a specificity of 94.9% (95% CI: 92.7–97.1) (13). It might depend on the study population or syphilis stages. Our results showed the highest sensitivity and specificity compared to the results of any of the previous multicenter reports (13). One report from the Reproductive Health and Research Department of WHO comparatively evaluated six different syphilis rapid diagnostic tests by using TPHA assay as a reference; SD Bioline Syphilis 3.0 showed 100% sensitivity and specificity, similar to the result of our study (14). In addition, no false-positive reactions due to the presence of ANAs or samples from pregnant women were observed in our study. There was only a single case of discrepant result between SD Bioline 3.0 and TPPA assays. The discrepant case produced a negative result of the rapid SD Bioline 3.0 assay and a positive result of the TPPA assay, and the FTA-ABS test showed minimally reactive. These discrepant results were obtained in a sample from a 61-year-old male patient who had dermatological problems with no syphilis history confirmed by his dermatologist. Therefore, we considered that this patient was not infected with syphilis and TPPA, and FTA-ABS results were dual false positive. Serological false-positive results of syphilis test are common and have been documented in the past (15–20). The main causes of false-positive results included systemic lupus erythematosus (15), diabetes mellitus (16), HIV infection (17, 18), leprosy (19), and prozone phenomenon (20). We did not notice any definite false interference of the rapid ICT used in our study. However, false-positive results of the treponemal tests were reported as often transient, and the underlying causes have not been fully understood (8). In United States and Europe, several recent reports have suggested that treponemal-specific tests should be used as the first-line serologic screening test of syphilis for their high sensitivity and specificity (5–7). However, Centers for Disease Control and Prevention (CDC) still have recommended screening with a nontreponemal test such as RPR test (4). Unfortunately, in this study, we could not categorize the patients according to the syphilis stages. Treponemal tests can vary in their reactivity in early primary syphilis, but in secondary and latent stages of syphilis the treponemal tests are usually 100% reactive (8).

In conclusion, SD Bioline Syphilis 3.0 showed an excellent performance compared to the TPPA assay. It can be suggested that this rapid ICT can be applied in clinical settings considering a simple test procedure, and rapid result with comparable performance to the previous treponemal tests.

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