Original Article

# Evaluation of SD BIOLINE Syphilis 3.0 for Rapid Diagnosis of Syphilis: Report from a Regional Sexually Transmitted Infection Reference Laboratory in North India

**Bhanu Mehra, Sonali Bhattar, Shikhar Saxena, Deepti Rawat, Preena Bhalla**

Department of Microbiology, Maulana Azad Medical College, New Delhi, India

**Address for correspondence:** Dr. Preena Bhalla, E-mail: preenabhalla@gmail.com

## ABSTRACT

**Background:** Serology is considered the mainstay of syphilis diagnosis. The limitations of the traditional serological methods and the advent and availability of novel immunochromatographic assays have led to the widespread application of rapid point-of-care procedures as screening tools for syphilis. However, these tests have not been extensively evaluated. This study was designed to evaluate the performance of a rapid syphilis diagnostic test known as SD BIOLINE Syphilis 3.0 (SD Biostandard Diagnostics Private Limited, Gurgaon, Haryana, India).

**Materials and Methods:** A panel comprising of 50 venereal disease research laboratory reactive and 50 nonreactive sera was tested using SD BIOLINE Syphilis 3.0. The performance of the test was evaluated using IMMUTREP *Treponema pallidum* hemagglutination assay (TPHA) (OMEGA Diagnostics Limited, Scotland, United Kingdom) as the reference standard and sensitivity, specificity, and negative and positive predictive values were calculated.

**Results:** The sensitivity, specificity, and positive and negative predictive values of SD BIOLINE Syphilis 3.0 were 92.86% (confidence interval of 95%: 80.52–98.50%), 98.28% (90.76-99.96%), 97.50% (86.84–99.94%), and 95.00% (86.08–98.96%), respectively, compared to TPHA as the gold standard.

**Conclusion:** Keeping in view the high sensitivity and specificity of SD BIOLINE Syphilis 3.0, we conclude that the test can be used as a tool for rapid on-site diagnosis of syphilis and as an alternative to TPHA for detection of antibodies to *Treponema pallidum*.

**Key words:** Non-treponemal tests, rapid, serodiagnosis, syphilis, treponemal tests

## INTRODUCTION

Syphilis is a sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum (T. pallidum)*. It is a common STI in India.[1,2] The inability to culture the causative organism *in vitro* and the limited availability of nucleic acid amplification techniques makes the diagnosis of this infection difficult. Moreover, direct visualization of the organism does not seem to be feasible since it mandates the presence of lesions and of facilities with either dark field or fluorescent microscopy.[3] Serology is thus considered the mainstay of syphilis diagnosis.

Serodiagnosis of syphilis relies on detection of two types of antibodies-antibodies against the cardiolipin antigen, and the treponema-specific antibodies.[4,5] A major diagnostic limitation encountered with the use of anticardiolipin antibody-based tests

Access this article online

Quick Response Code:

Website: www.jlponline.org

DOI: 10.4103/0974-2727.176239



**How to cite this article:** Mehra B, Bhattar S, Saxena S, Rawat D, Bhalla P. Evaluation of SD BIOLINE Syphilis 3.0 for rapid diagnosis of syphilis: Report from a regional sexually transmitted infection reference laboratory in North India. J Lab Physicians 2016;8:36-40.

(nontreponemal tests) is the occurrence of biological false positive (BFP) reactions.[6-8] It is, therefore, recommended to use nontreponemal tests such as venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR) test as screening assays followed by confirmation of the nontreponemal reactivity by the more specific treponemal tests like *T. pallidum* hemagglutination assay (TPHA) and fluorescent treponemal antibody absorption test.[9-11] False negative reactions due to the prozone phenomenon are also seen with nontreponemal tests.[12] Moreover, the tests lack sensitivity in the late latent stage of infection.[13]

A major drawback of the laboratory procedures currently in use for syphilis serodiagnosis is that they require laboratory facilities (refrigeration, water bath, centrifuge, rotator, etc.); stringent quality control measures and skilled persons to perform the tests, as well as trained health professionals to read and interpret the results. In resource constraint settings, laboratory infrastructure and facilities for syphilis diagnosis might not be widely available, and the delay encountered in getting the samples tested from referral laboratories may preclude timely initiation of treatment. This eventually translates into continued transmission of disease to the naive or uninfected individuals.

The current situation mandates the need for rapid and reliable tests to serve as screening and confirming assays in all stages of syphilis. Rapid serological procedures offer a potential option with assured rapid availability of results usually in <15 min and ease of use by health professionals allowing on-site testing. The World Health Organization Sexually Transmitted Diseases Diagnostic Initiative has laid down the ASSURED criteria that define the ideal characteristics of a rapid and point-of-care test: Affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to those who need them.[14-16] Several rapid, point-of-care assays based on recombinant *T. pallidum* antigens are now commercially available.[17] Despite the benefits that the rapid tests offer over traditional laboratory methods for syphilis serodiagnosis, their diagnostic performance remains a matter of concern and is still not widely documented.

In this study, the authors have evaluated the performance of SD BIOLINE Syphilis 3.0 (SD Biostandard Diagnostics Private Limited, Gurgaon, Haryana, India), a rapid immunochromatographic assay that qualitatively detects antibodies against *T. pallidum*. To the best of our knowledge, there is no published peer-reviewed article in medical literature regarding the evaluation of this test kit in an Indian scenario.

## MATERIALS AND METHODS

This prospective study was conducted at the Department of Microbiology of a tertiary care health facility in New Delhi. The study was duly approved by the Institutional Review Board. The serology laboratory of the department receives on an average 15,000 serum samples annually for syphilis serology from both the Inpatient and Outpatient Departments of the linked hospitals. Consecutive blood samples received at the serology laboratory from October 2013 to February 2014 with request for syphilis screening were included in the analysis. On arrival in the laboratory, the samples were allowed to coagulate and centrifuged at 2500 rpm (rotations per minute) for 10 min. The sera were separated and stored at 2–8°C till VDRL (employing antigen from Serologist to Government of India, Kolkata) was put up (generally every 2$^{nd}$ or 3$^{rd}$ day). All VDRL reactive sera were serially diluted to determine the antibody titre. After being tested as per the above protocol, the sera were aliquoted and stored in eppendorfs at − 20°C until a panel of 100 sera comprising of 50 VDRL reactive and 50 nonreactive samples were reached. A different technician who did not have access to results of the screening assay, performed the IMMUTREP TPHA (OMEGA Diagnostics Limited, Scotland, United Kingdom) as per the manufacturer's instructions in the kit insert. The third laboratory technician, who was again blinded to the VDRL and IMMUTREP TPHA results performed SD BIOLINE Syphilis 3.0 in accordance with the manufacturer's guidelines. The procedural details of the tests employed in this study along with their interpretative criteria are provided in Table 1 while their characteristics are summarized in Table 2.

The results of VDRL test, IMMUTREP TPHA, and SD BIOLINE Syphilis 3.0 were entered in Microsoft Excel sheet and any discordant results by IMMUTREP TPHA and SD BIOLINE Syphilis 3.0 were tested again by both the procedures before they were finally recorded as positive or negative. The sensitivity and specificity calculations and estimation of negative and positive predictive values of SD BIOLINE Syphilis 3.0 were done by comparing its performance with IMMUTREP TPHA (reference technique). These values were calculated with their respective confidence interval of 95% (95% CI). Sensitivity was calculated as true positives/(true positives + false negatives) ×100; specificity as true negatives/(true negatives + false positives) ×100; negative predictive value as true negatives/(true negatives + false negatives) ×100, and positive predictive value as true positives/(true positives + false positives) ×100.

**Table 1: Methodology and interpretation of tests used for serological diagnosis of syphilis**

| VDRL* | IMMUTREP TPHA† | SD BIOLINE Syphilis 3.0 |
|---|---|---|
| | **Procedure** | |
| Sera inactivated by heating at 56°C for 30 min | Dispense 190 µL of diluent into row 1 | Remove test device from foil pouch |
| 50 µL of heat inactivated serum allowed to react with 1/60 ml of VDRL antigen emulsion on the slide | Dispense 10 µL of sample to row 1 and mix | Add 10 µL serum/plasma or 20 µL whole blood to the sample well |
| Rotated at 180 rpm‡ for 4 min | Discard 150 µL from row 1 | Add 3-4 drops of assay diluent |
| Results immediately read under a microscope with low power objective by a medical microbiologist with at least 3 years training in microbiology | Add 25 µL from row 1 to row 2 | Interpret test results at 5-20 min for serum/plasma and within 10 min for whole blood |
| | Add 75 µL of well-mixed test cells to row 1 | |
| | Add 75 µL of well-mixed control cells to row 2 | |
| | Tap plate gently to mix | |
| | Cover and let stand at room temperature for 45 to 60 minutes | |
| | Examine for agglutination patterns | |
| | **Interpretation of the test** | |
| Nonreactive: No clumps or very slight roughness | Negative result: Absence of agglutination | Negative result: Presence of only one purple color band within the result window |
| Weakly reactive: Small clumps | Positive result: Agglutination of the test cells but not the control cells | Positive result: Presence of both "T" band and "C" band within the result window |
| Reactive: Medium and large clumps | Invalid result: Agglutination of the control cells as well as the test cells | Invalid result: No purple color band visible within the result window |

*VDRL: Venereal disease research laboratory, †TPHA: *Treponema pallidum* hemagglutination assay, ‡rpm: Rotations per minute

**Table 2: Characteristics of tests used for serological diagnosis of syphilis**

| Test | Principle | Antigen employed | Specimen type | Specimen volume required | Time to test result |
|---|---|---|---|---|---|
| VDRL* | Slide flocculation | An alcoholic solution of cardiolipin (0.03%), purified lecithin (0.21%) and cholesterol (0.9%) | Serum, CSF‡ | 50 µL | Test read immediately after rotation |
| IMMUTREP TPHA† | Passive hemagglutination | *T. pallidum* antigen | Serum, CSF‡ | 10 µL | 45-60 min |
| SD BIOLINE Syphilis 3.0 | Solid phase immunochromatographic assay (lateral flow) | 17 kDa and 15 kDa recombinant *T. pallidum* antigens | Serum, plasma | 10 µL | 5-20 min |
| | | | Whole blood | 20 µL | Within 10 min |

*VDRL: Venereal disease research laboratory, †TPHA: *Treponema pallidum* hemagglutination assay, ‡CSF: Cerebrospinal fluid, *T. pallidum: Treponema pallidum*

## RESULTS

Of the 50 VDRL reactive samples, 42 (84%) were TPHA positive, and 40 (80%) were reactive with SD BIOLINE Syphilis 3.0. Considering TPHA as the gold standard, the 8 VDRL reactive and TPHA negative samples can be considered as BFPs. All but one of these 8 BFP samples was correctly identified as negative by SD BIOLINE Syphilis 3.0. In addition, of the 42 true positives picked up by IMMUTREP TPHA, 3 were wrongly identified as negative by SD BIOLINE Syphilis 3.0 and remained so on repeat testing. Two of the samples with false-negative rapid test results had a VDRL titre of 1:2, and one sample had a VDRL titre of 1:16. All the VDRL nonreactive sera ($n$ = 50) were negative by both IMMUTREP TPHA and the rapid test. The performance of SD BIOLINE Syphilis 3.0 and VDRL as against IMMUTREP TPHA is presented in Tables 3 and 4, respectively.

Compared to IMMUTREP TPHA as the gold standard, the sensitivity, specificity, and positive and negative predictive values of SD BIOLINE Syphilis 3.0 were 92.86% (95% CI: 80.52–98.50%), 98.28% (90.76–99.96%), 97.50% (86.84–99.94%), and 95.00% (86.08–98.96%), respectively. The kappa value was 0.917, showing a "very good" strength of agreement between the two tests. The performance characteristics of the currently employed VDRL test in comparison to the gold standard (IMMUTREP TPHA) are as follows: Sensitivity = 100.00% (91.59–100.00%), specificity = 86.21% (74.62–93.85%), positive predictive value = 84.00% (70.89–92.83%), and negative predictive value = 100.00% (92.89–100.00%). Thus, while the sensitivity of SD BIOLINE Syphilis 3.0 was lower than that of VDRL (92.86% vs. 100.00%), the specificity was much higher (98.28% vs. 86.21%).

## DISCUSSION

We report sensitivity and specificity values of 92.86% and 98.28%, respectively, for SD BIOLINE Syphilis 3.0, compared to TPHA as the reference standard. A study conducted in Tanzania found the test to be 79% sensitive and 96% specific.[18] In another study conducted in China, the reported sensitivity and specificity values were 95.5% and 97.9%, respectively.[19] Thus, keeping in view the high sensitivity and specificity of SD BIOLINE Syphilis 3.0, we conclude that the test can be used both as a screening assay and as an alternative to TPHA for detection of antibodies to *T. pallidum*. We found SD BIOLINE Syphilis 3.0 to be less sensitive but more specific than VDRL. Lower sensitivity of immunochromatographic tests has also been

**Table 3: Performance of SD BIOLINE Syphilis 3.0 in comparison with IMMUTREP TPHA* (reference standard) and VDRL†**

| Test | TPHA | | VDRL | |
|---|---|---|---|---|
| Result | Positive | Negative | Positive | Negative |
| SD BIOLINE Syphilis 3.0 | | | | |
| Positive | 39 | 1 | 40 | 0 |
| Negative | 3 | 57 | 10 | 50 |

*TPHA: *Treponema pallidum* hemagglutination assay, †VDRL: Venereal disease research laboratory

**Table 4: Performance of VDRL* in comparison with IMMUTREP TPHA† (reference standard)**

| Test | TPHA | |
|---|---|---|
| Result | Positive | Negative |
| VDRL | | |
| Positive | 42 | 8 |
| Negative | 0 | 50 |

*VDRL: Venereal disease research laboratory; †TPHA: *Treponema pallidum* hemagglutination assay

reported in previous studies, and the reason cited has been primarily the low levels of antibodies found in sera of patients with early primary syphilis.[20]

The discrepant results between IMMUTREP TPHA and SD BIOLINE Syphilis 3.0 (IMMUTREP TPHA positive and SD BIOLINE Syphilis 3.0 negative sera) can be explained by the fact that a large number of treponemal antigens are exposed during the TPHA test. Therefore, some cross reaction with other organisms may occur. Moreover, as has been hypothesized by some researchers, though the recombinant antigens employed in the rapid tests are specific to *T. pallidum*, posttranslational processing of these protein *in vivo* or genetically determined unresponsiveness or restricted responsiveness to these antigens could lead to lack of reactivity even in the presence of disease.[21]

SD BIOLINE Syphilis 3.0 is a rapid, one-step test that is easy to execute, read, and interpret. Apart from serum samples, the test is also compatible with whole blood and plasma. Therefore, neither does the test require any preprocessing of specimens nor does it require any laboratory infrastructure or trained technical personnel for performing the test procedure. This makes the test an ideal on-site screening option in resource-constraint health-care settings without laboratory facilities. In addition, since the test detects treponema-specific antibodies, BFPs do not occur and the test can also be used as an alternative to TPHA for confirming the reactivity in nontreponemal assays. The rapid availability of results with the use of this test would also ensure prompt treatment of reactive patients in their very first visit to the health care services.

The test, however, is fraught with certain shortcomings. An important constraint to its application as a screening tool is that the treponema-specific antibodies detected by the test are retained for years and thus it cannot distinguish past treated infections from recent or active ones.[22] This implies that a large number of previously treated infections would also be picked up by SD BIOLINE Syphilis 3.0, particularly in high-prevalence settings, thereby leading to unnecessary treatment of patients. The test can also give a positive result in various nonvenereal treponematoses such as yaws and pinta.[23] However, some researchers argue that false positive results are preferable to false negative ones.[24] While with a false negative result, a syphilitic patient may go untreated and transmit the infection to others; a false positive serology would at least trigger repeat testing with alternative methods before a definitive diagnosis is made. In this context, we also suggest adopting a reverse algorithm, whereby a nontreponemal assay such as VDRL or RPR could be used to document active disease in patients with a reactive point-of-care treponemal test. Novel dual rapid point-of-care tests have also been devised where both treponemal and nontreponemal tests are combined into one device and can thus simultaneously serve as screening, as well as confirmatory assays.[25] However these tests are still under evaluation and not available for routine use.

Our study has a great relevance from public health perspective and based on our findings we recommend the use of rapid point-of-care procedures such as SD BIOLINE Syphilis 3.0 as primary screening assays for serodiagnosis of syphilis where laboratory facilities are not available and as tests to confirm nontreponemal reactivity where TPHA cannot be done. The study was conducted at a regional STI reference laboratory located at a tertiary care center in North India. The laboratory screens a large number of samples from in-patient and out-patient sections of various departments of the linked hospitals and thus caters to a diverse population base.

This study has a few limitations. First, the number of sera in the panel used for kit evaluation was small. In addition, ours was a laboratory-based study and the evaluation was not done under field or clinical conditions for which the test is primarily intended. Moreover, the test kit was exclusively evaluated using serum samples and its performance characteristics with whole blood and plasma specimens were not determined. Also, we did not have clinical information of the cases from which the reactive sera were obtained and, therefore, performance of the test according to the stage of syphilis could not be assessed.

## CONCLUSION

To summarize, we strongly recommend the use of SD BIOLINE Syphilis 3.0 for rapid on-site diagnosis of syphilis. Further field studies are required before the test can be routinely implemented as a screening assay.


### Financial support and sponsorship

Nil.


### Conflicts of interest

There are no conflicts of interest.

## REFERENCES


1. Mohanty J, Das KB, Mishra C. Clinical profile of sexual transmitted diseases in Cuttack. Indian J Dermatol Venereol Leprol 1995;61:143-4.
2. Ray K, Bala M, Gupta SM, Khunger N, Puri P, Muralidhar S, *et al.* Changing trends in sexually transmitted infections at a Regional STD Centre in North India. Indian J Med Res 2006;124:559-68.
3. Seña AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: A paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010;51:700-8.
4. Allen RH, Mason MA. Cardiolipin antigens in the serodiagnosis of syphilis. Can J Public Health 1952;43:128-35.
5. Gerber A, Krell S, Morenz J. Recombinant *Treponema pallidum* antigens in syphilis serology. Immunobiology 1996-1997;196:535-49.
6. Rusnak JM, Butzin C, McGlasson D, Blatt SP. False-positive rapid plasma reagin tests in human immunodeficiency virus infection and relationship to anti-cardiolipin antibody and serum immunoglobulin levels. J Infect Dis 1994;169:1356-9.
7. Nandwani R, Evans DT. Are you sure it's syphilis? A review of false positive serology. Int J STD AIDS 1995;6:241-8.
8. Bernard C, de Moerloose P, Tremblet C, Reber G, Didierjean L. Biological true and false serological tests for syphilis: Their relationship with anticardiolipin antibodies. Dermatologica 1990;180:151-3.
9. Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. Nat Rev Microbiol 2004;2:231-40.
10. Young H. Syphilis: New diagnostic directions. Int J STD AIDS 1992;3:391-413.
11. Centers for Disease Control and Prevention (CDC). Discordant results from reverse sequence syphilis screening – five laboratories, United States, 2006-2010. MMWR Morb Mortal Wkly Rep 2011;60:133-7.
12. Berkowitz K, Baxi L, Fox HE. False-negative syphilis screening: The prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy. Am J Obstet Gynecol 1990;163:975-7.
13. Ebel A, Bachelart L, Alonso JM. Evaluation of a new competitive immunoassay (BioElisa Syphilis) for screening for *Treponema pallidum* antibodies at various stages of syphilis. J Clin Microbiol 1998;36:358-61.
14. Peeling RW, Mabey D, Herring A, Hook EW 3rd. Why do we need quality-assured diagnostic tests for sexually transmitted infections? Nat Rev Microbiol 2006;4:909-21.
15. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 2010;16:1062-9.
16. Mabey DC, Sollis KA, Kelly HA, Benzaken AS, Bitarakwate E, Changalucha J, *et al.* Point-of-care tests to strengthen health systems and save newborn lives: The case of syphilis. PLoS Med 2012;9:e1001233.
17. Wang LN, Yang L, Zheng HY. Clinical evaluation of four recombinant *Treponema pallidum* antigen-based rapid tests in the diagnosis of syphilis. Chin Med Sci J 2007;22:250-3.
18. Aboud S, Lyamuya EF, Sufi J, Haule E. Comparison of the performance of SD Bioline Syphilis 3.0 assay with the RPR test for the syphilis screening in Dar es Salaam, Tanzania. Tanzania Med J 2006;21:6-7.
19. Li J, Zheng HY, Wang LN, Liu YX, Wang XF, Liu XR. Clinical evaluation of four recombinant *Treponema pallidum* antigen-based rapid diagnostic tests for syphilis. J Eur Acad Dermatol Venereol 2009;23:648-50.
20. Sato NS, de Melo CS, Zerbini LC, Silveira EP, Fagundes LJ, Ueda M. Assessment of the rapid test based on an immunochromatography technique for detecting anti-*Treponema pallidum* antibodies. Rev Inst Med Trop Sao Paulo 2003;45:319-22.
21. West B, Walraven G, Morison L, Brouwers J, Bailey R. Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. Sex Transm Infect 2002;78:282-5.
22. Centers for Disease Control and Prevention (CDC). Syphilis testing algorithms using treponemal tests for initial screening – Four laboratories, New York City, 2005-2006. MMWR Morb Mortal Wkly Rep 2008;57:872-5.
23. Ratnam S. The laboratory diagnosis of syphilis. Can J Infect Dis Med Microbiol 2005;16:45-51.
24. Lien TX, Tien NT, Chanpong GF, Cuc CT, Yen VT, Soderquist R, *et al.* Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg 2000;62:301-9.
25. Castro AR, Esfandiari J, Kumar S, Ashton M, Kikkert SE, Park MM, *et al.* Novel point-of-care test for simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. J Clin Microbiol 2010;48:4615-9.