Original Article

Does performance and operational superiority of point-of-care test make it the investigation of choice in confirming syphilis?

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Abstract

Background: According to the World Health Organization, 6 million cases of syphilis occur every year. Serological tests for syphilis form the mainstay of diagnosis for syphilis. We evaluated the performance of point-of-care test (POCT) against other specific treponemal test for confirming the diagnosis of syphilis. **Materials and Methods:** Does performance and operational superiority of POCT make it the investigation of choice in confirming syphilis? Retrospectively, data were analyzed of 599 serum samples from Apex Regional sexually transmitted disease centre, Safdarjung Hospital, New Delhi, received for testing by syphilis treponemal assays (both nontreponemal reactive and nonreactive). These samples underwent treponemal testing for syphilis by the *Treponema pallidum* hemagglutination (TPHA), fluorescent treponemal antibody absorption test (FTA-ABS), and POCT. Performance characteristics (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], and diagnostic accuracy), and operational characteristics of POCT and TPHA were evaluated against the gold standard FTA-ABS. **Results:** A total of 599 samples were evaluated, of which 61.76% were positive by FTA-ABS. On analysis, the sensitivity was 91.08% and 91.89%, specificity was 89.08% and 87.34%, PPV was 93.09% and 92.14%, NPV was 86.08% and 86.96%, and diagnostic accuracy was 90.32% and 90.15% for POCT and TPHA, respectively. The lower cost, shorter turnaround time, lesser infrastructure and workforce need, and easy availability make the POCT operationally superior to TPHA. **Conclusion:** Owing to its operational superiority and higher specificity POCT can replace TPHA for confirming the diagnosis of Syphilis. POCT are affordable, equipment free, have room temperature storage, and yield result within 15 minutes, enabling same day testing and treatment. It can be used in a resource limited setting, for community setup or even self-testing.

Key words: Fluorescent treponemal antibody absorption, point-of-care test, syphilis, *Treponema pallidum* hemagglutination

Introduction

The global burden of sexually transmitted infections (STIs) is high. In 2016, an estimated 376 million new infections (more than 1 million per day) of the four STIs – chlamydia, gonorrhea, syphilis, and trichomoniasis – were reported.[1] According to the WHO, 7 million new syphilis infections were documented in 2020[2] of which low-income countries constitute 90% burden of the disease. Furthermore, 300,000 fetal and neonatal deaths are attributable to syphilis, whereas 215,000 additional infants are subjected to an increased risk of early death.[3]

Syphilis is a preventable and curable STI caused by spirochete *Treponema pallidum*. It is a major STI in men and has been challenging clinicians since time immemorial due to its variable clinical course.[4] Diagnostic tests for syphilis are divided into direct and indirect tests;[5] direct diagnostic methods include dark ground microscopy wherein tissue fluids are examined as a wet mount using dark-field microscopy.[5] Although this test is useful in patients with immunodeficiency or in early syphilis when antibodies are not detectable, it requires a trained, experienced microscopist and is dependent on a number of factors, including the amount of fluid on the slide, improper thickness of the slide or coverslip, etc. Therefore, while *T. pallidum* demonstration is the gold standard for diagnosis, dark-field microscopy has poor sensitivity, and failure to identify *T. pallidum* by this test

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does not rule out syphilis. Another direct test, polymerase chain reaction (PCR) is highly sensitive and has potential as a test of choice for congenital syphilis, neurosyphilis, early primary syphilis[6] but lack commercially accessible PCR-based test kits, specialized infrastructure and skilled workforce. Indirect diagnostic tests also known as serological tests are divided into treponemal and nontreponemal tests.[7] Nontreponemal test consisting of the Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR) are typically thought to be sensitive in the early stages of syphilis, but they have the drawback of false-positive reactions due to cross-reactivity with autoimmune disorders, collagen diseases, and infections including malaria and leprosy. Due to its diminished sensitivity in primary syphilis and late latent syphilis, they also have false-negative results. Hence, in the classical testing strategy being followed in India, nontreponemal tests are used for screening of syphilis patients, followed by the confirmation by treponemal assays. Treponemal assays are specific for *T. pallidum* antibodies and consist of *T. pallidum* hemagglutination assay (TPHA), *T. pallidum* particle agglutination assay (TPPA), fluorescent treponemal antibody absorption (FTA-ABS), treponemal enzyme immunoassay (EIA), and other point-of-care tests (POCT) immunochromatographic strip assays.

Diagnostic tests for syphilis have been unavailable in many resource-constrained situations, as they required technical skill, equipment, and energy. POCTs, which do not require equipment, can be stored at room temperature, and provide a result in 15 min, are now available, allowing syphilis to be diagnosed at the point of care rather than in a laboratory. Patients do not need to return for their results because a diagnosis can be made at the initial appointment, and treatment can be started right away, enabling "same-day testing and treatment" (STAT).[8]

The characteristics of an ideal POCT are affordability, high sensitivity and specificity, user-friendliness, rapid and robust in providing treatment at the first visit, free of equipment, and can be delivered to those who need it, i.e., the ASSURED criteria.[9] Most of the available POCTs fulfill the above-mentioned characteristics, hence, providing testing facilities at lower levels of health-care system and enabling a better coverage of syphilis screening in pregnancy.

Thus, the aim of our study is to evaluate the performance of POCT and to compare it with TPHA using FTA-ABS as the gold standard for confirming the diagnosis of syphilis.

Materials and Methods

Study site

The study was conducted at the apex regional sexually transmitted disease (STD) Centre and the Department of Dermatology, Venereology, and leprosy, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. Being a tertiary reference laboratory, samples are received routinely from STIs clinic, antenatal patients, and other attendees for screening of syphilis.

Study samples

The samples were screened by a nontreponemal test for syphilis, namely, VDRL. Those positive and a few negatives, where a treponemal assay had been requested, were stored in a refrigerator and were then confirmed weekly by FTA-ABS, TPHA, and POC test. A total of 599 serum samples were obtained from 395 male patients and 204 female patients tested during the study.

Venereal disease research laboratory

For VDRL testing (the Institute of Serology, Kolkata, India), a 7 ml venous blood sample was collected in a plain vacutainer, allowed to clot for 15 min, and centrifuged for 10 min at 2000 g. The VDRL antigen was prepared as per the manufacturer's recommendations. The flocculation test was performed on glass slides, and the semiquantitative results were observed under a microscope. Known positive and negative samples were used as controls with each run.

Treponema pallidum hemagglutination

The TPHA test was a standard Immutrep test (Omega Diagnostics Ltd., United Kingdom). Sera were diluted and mixed with *T. pallidum* sensitized formalized tanned fowl erythrocytes, antibody to the sensitizing antigen if present in the serum caused agglutination of the cells. The cells formed a characteristic pattern of cells in the bottom of a microtiter plate well and the results were read as positive. In the absence of antibody, they formed a compact button in the well, and the results were read as negative. Positive control, negative control, and antigen control were included with each test run.

Fluorescent treponemal antibody absorption

The FTA-ABS test kit (Bioscientifica) contained cultured organisms placed in wells of specially prepared microscopic slides. Sorbent-treated sera were placed on antigen-coated wells where antibody if present bound to the antigen. The reaction was visualized using a fluorescein-labeled, antihuman globulin. The conjugate is bound with human antibodies attached to the cells causing them to fluoresce when viewed through a microscope equipped with ultraviolet light source. Since the antigen was composed of intact bacterial cells, the fluorescent image through the microscope consisted of spirochaetes.

Point-of-care test

The rapid POCT test devices for syphilis serology (Medsource Ozone Biomedicals Pvt. Ltd., Haryana, India) consisted of an immunochromatographic strip (ICS) coated with highly purified recombinant antigens. The serum was added at one end of the strip. Clear dark bands for the control and test line indicated a positive test result.

Quality assurance

The laboratory at Apex Regional STD Centre is the Apex Reference Laboratory for STI Prevention and Control Program. It has been participating successfully in the Syphilis Serology Proficiency Testing Scheme conducted by the Centers for Disease Control and Prevention (CDC) for the past 10 years for all the serological tests for syphilis. The laboratory is also National Accreditation Board for Testing and Calibration Laboratories accredited for all its syphilis serological tests as per laboratory-based *Treponema Pallidum*-specific (ISO) 15189:2021 standards.

Statistical analysis

The data were arranged, and test characteristics such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy of POC test, and TPHA were evaluated against the gold standard FTA-ABS.

RESULTS

Upon performing the nontreponemal screening assay on 599 serum samples, a total of 319 VDRL reactive and 280 VDRL nonreactive serum samples were obtained from 395 male patients and 204 female patients.

When the results were confirmed by treponemal specific tests, 61.76% were positive by FTA-ABS, 61.60% were positive by TPHA, and 60.43% were positive by POC test [Table 1].

Performance characteristics

The sensitivity, specificity, diagnostic accuracy, and predictive values of TPHA and POC test in this study were evaluated using FTA-ABS as a gold standard for the diagnosis of syphilis. Upon analysis, the sensitivity of POC test was 91.08% (95% confidence interval [CI]: 87.70%–93.78%) and TPHA was 91.89% (95% CI: 88.63%–94.46%); specificity of POC test was 89.08% (95% CI: 84.31%–92.81%) and TPHA was 87.34% (95% CI: 82.32%–91.35%); PPV of POC test was 93.09% (95% CI: 89.97%–95.48%) and TPHA was 92.14% (95% CI: 88.91%–94.67%); NPV of POC test was 86.08% (95% CI: 81%–90.22%) and TPHA was 86.96% (95% CI: 81.91%–91.02%); and diagnostic accuracy of POC test was 90.32% and TPHA was 90.15%. Even though sensitivity, NPV, and diagnostic accuracy of POC test were comparable with TPHA, the specificity and PPV of POC test were higher than that of TPHA; characteristics which are important for a confirmatory assay [Table 2].

Table 1: Comparison of gold standard fluorescent treponemal antibody absorption test with *treponema pallidum* hemagglutination and point of care test results

	FTA-ABS		Total
	Negative	Positive	
TPHA			
Negative	200	30	230
Positive	29	340	369
POCT			
Negative	204	33	237
Positive	25	337	362
Total	229	370	599

FTA-ABS=Fluorescent treponemal antibody absorption; TPHA=*Treponema pallidum* hemagglutination; POCT=Point-of-care test

Table 2: Comparison of performance characteristics of treponemal tests

Values	TPHA (95% CI)	POCT (95% CI)
Sensitivity	91.89 (88.63-94.46)	91.08 (87.70-93.78)
Specificity	87.34 (82.32-91.35)	89.08 (84.31-92.81)
PPV	92.14 (88.91-94.67)	93.09 (89.97-95.48)
NPV	86.96 (81.91-91.02)	86.08 (81-90.22)
Diagnostic accuracy	90.15	90.32

PPV=Positive predictive value; NPV=Negative predictive value; TPHA=*Treponema pallidum* hemagglutination; POCT=Point-of-care test; CI=Confidence interval

Operational characteristics

Upon comparing the operational characteristics of POC test and TPHA, POC test was found to be worthier as it was easy to perform and did not need any training. The test can be performed by peripheral health-care workers. The results are available within 15 min which makes it possible to treat the patient at the same visit. Room temperature storage of the POC test kits ensures that the kit can be used in rural areas where refrigerators may not be available. Moreover, the POCT was at least half the cost of TPHA test (ignoring the cost of the micropipettes and other consumables). POC test is manufactured locally and can be easily procured with less lead time, whereas TPHA assays are not manufactured locally and have to be imported [Table 3].

Discussion

According to the WHO, 6 million active cases of syphilis occur every year, and among women of childbearing age, the highest burden is in Sub-Saharan Africa.[10] The seroprevalence of syphilis in pregnant women was reported as 0.38%, and the annual burden of syphilis among pregnant women in India is roughly 103,960, out of which 53,187 show any poor outcomes, including 21,488 early fetal loss/stillbirths, 9213 neonatal deaths, 6161 premature or low birth weight babies, and 16,324 newborns displaying clinical evidence of syphilis.[11] These adverse pregnancy outcomes can be prevented by a single dose of long-acting benzathine penicillin given before 28 weeks' gestation.[12] Therefore, early diagnostic interventions form the mainstay in controlling syphilis and preventing its adverse outcomes, especially in patients with no clinical signs and symptoms.

Treponemal assays used for the diagnosis of syphilis are more specific and consist of TPHA, TPPA, FTA-ABS, EIA, and other POCTs. Tucker *et al*. conducted a systematic analysis of 15 studies evaluating syphilis POC testing, totaling 23,055 individual test results. Thirteen of the studies were conducted in antenatal care or STI clinic settings in low- or middle-income countries. The ICS test sensitivity was found to be 86% on average (interquartile range 75%–94%). The syphilis ICS tests' specificity varied from 90.9% to 100.0%, with a median of 99%.[13] In a study conducted by Bronzan *et al*. in 2007, eight rural clinics performed the on-site RPR and ICS tests, and the results were compared with RPR/TPHA at a reference laboratory.[14] It was found that 79 (6.3%) of 1250 women screened on-site had active syphilis according to the reference laboratory. The on-site ICS resulted in the

Table 3: Comparison of operational characteristics of treponemal tests

Operational characteristics					
TPHA	50 INR plus consumables	1 h	At least trained laboratory technician	Requires refrigerated storage	Needs to be imported
POCT	25 INR or less	10-15 min	Trained peripheral health worker or self-testing	Refrigerated storage is not required	Locally manufactured

TPHA=*Treponema pallidum* hemagglutination; POCT=Point-of-care test

highest percentage of pregnant women correctly diagnosed and treated for syphilis (89.4% ICS, 63.9% on-site RPR, and 60.8% offsite RPR/TPHA). Overall, rapid and POC tests performed well in sensitivity and specificity as compared to laboratory-based TP-specific tests such as TPPA and TPHA that have sensitivity in the range of 85%–100% and specificity in the range of 98%–100%.[15] Our study compared POC test and TPHA for their diagnostic characteristics to gold standard FT-ABS. In our study, the sensitivity and specificity of POC test were found to be 91.08% and 89.08%, whereas for TPHA it was 91.89% and 87.34%, respectively. Specificity and diagnostic accuracy of POCTs were higher as compared to TPHA, making it an ideal replacement for not only screening but also confirming syphilis. This is in concordance with the results of a study by Jafari *et al*.,[16] where POC test was performed using whole blood or serum specimens, and sensitivities ranged from 74% to 99% and specificities from 94% to 99%. The Alere Determine assay employing serum was the best-performing test in that study, with a sensitivity of 90% and a specificity of 94%.

However, traditional treponemal testing is still difficult in low-income countries disproportionately impacted by syphilis due to a reliance on skilled laboratory employees and tertiary laboratory facilities. These operational characteristics of diagnostic tests not only have a bearing on performance characteristics but also are most important when a test is supposed to be utilized in a resource-limited setup. With poor infrastructure, even simple laboratory activities such as cold storage, training of workforce, reading of results under a microscope, and cost can become a major challenge for implementation. In comparison, POCT is a rapid immunochromatographic test which uses whole blood or serum and yield result within 15 min. A positive test is marked as a colored line or dot in the membrane, and thus the treatment can be facilitated the same day. POC test is more affordable, robust, and free of equipment does not require refrigeration and can be delivered to those who need it.[9]

During the last couple of years, procurement of items which were to be imported was very difficult with unduly high lead time due to the international restrictions due to COVID-19 pandemic. This made procurement of TPHA tests in India difficult for most of the laboratories performing these assays. POCT was locally manufactured and easily available. The performance of this test was also 100% in the CDC External Quality Assurance Scheme activities in which Apex STD Centre participated and, in turn, conducted for multiple laboratories in the country. This confirms that the results of POC assay are accurate, reliable, and reproducible.

Conclusion

Owing to its operational superiority and higher specificity, POCT can replace TPHA for confirming the diagnosis of syphilis. POCT is affordable, equipment free, have room temperature storage, and yield result within 15 min, enabling STAT. It can be used in a resource-limited setting, for community setup or even self-testing.

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Conflicts of interest

There are no conflicts of interest.

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