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Evaluation of Rapid Treponemal Test For Serodiagnosis of Syphilis

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ABSTRACT

Objective: To evaluate the diagnostic accuracy of a commercial treponemal immunochromatographic test (ICT) R-test Syphilis Ab Rapid test, using positivity on two treponemal tests: Treponema pallidum haemagglutination assay (TPHA) and syphilis enzyme-linked immunosorbent assay (ELISA) as reference method for serodiagnosis of syphilis, as per European Center for Disease Prevention and Control (ECDC) algorithm.

Study Design: Cross sectional validation study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from Jul to Dec 2023.

Methodology: A total of 52 samples were analyzed, where samples detected positive on both TPHA and ELISA were considered as true positives while samples testing negative by both TPHA and ELISA were considered as true negatives. Results of ICT were then compared to these. Data were incorporated in Microsoft (MS) Excel and then analyzed using Statistical Package for Social Sciences (SPSS) version 26.00.

Results: Out of 55 collected samples, 52 were analyzed of which 27(52.00%) samples were positive and 24(46.00%) were negative by both the ICT method and the ELISA/TPHA combination (ECDC algorithm) while 1(2.00%) was detected positive by ECDC approach and negative by ICT method. Therefore, ICT was found to have sensitivity, specificity, accuracy and Cohen's kappa coefficient of 96.43%, 100.00%, 98.07% and 0.96 respectively.

Conclusion: The treponemal ICT (R-test Syphilis Ab Rapid test) was found to be highly sensitive, specific and accurate test for serodiagnosis of syphilis, making it a reliable and rapid diagnostic tool.

Keywords: Algorithm, Enzyme Linked Immunosorbent Assay, Serodiagnosis, Syphilis

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INTRODUCTION

Syphilis is a sexually-transmitted infectious disease caused by the spirochete Treponema pallidum (T. pallidum).[1] In 2020, 7 million new syphilis cases worldwide were reported[2] while in Pakistan, the disease burden is 29.50%.[3] As it is very difficult to carry out culture of T. pallidum, the diagnosis of syphilis is made either with dark-field microscopy (DFM), polymerase chain reaction (PCR) or serological testing.[4] Serological testing consists of Non-Treponemal Tests (NTTs) including Venereal Disease Research Laboratory test (VDRL) and Rapid Plasma Reagin (RPR) and Treponemal Tests (TTs) including enzyme-linked immunosorbent assay (ELISA), T. pallidum hemagglutination assay (TPHA), T. pallidum particle agglutination assay (TPPA), immunochromatographic test (ICT), chemiluminescence immunoassay (CLIA) and fluorescent treponemal antibody-absorption (FTA-ABS) with the recommended algorithm by Centre for Disease Control and Prevention (CDC) starting from a TT and followed by a quantitative NTT, which if found negative, is confirmed by TPPA but according to the algorithm recommended by European Centre for Disease Prevention and Control (ECDC), diagnosis begins with a TT and a positive result is then confirmed by an alternate TT method.[5-8] If the confirmatory test is positive, a quantitative NTT is performed for the assessment of activity of disease and treatment response.[9] An accurate and early diagnosis of syphilis remains a challenge as CLIA and automated ELISA are accurate but require specialized equipment with training and are expensive while Rapid ICT is economical and simple to perform, hence, it was essential to evaluate the reliability of ICT so that it can serve as substitute of CLIA and ELISA in low-income settings. Therefore, this study was planned to evaluate the diagnostic accuracy of a commercial treponemal ICT, R-test Syphilis Ab Rapid test, using positivity on two treponemal tests (TPHA/ syphilis ELISA) as reference method for serodiagnosis of syphilis, as per ECDC algorithm.

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METHODOLOGY

This cross-sectional validation study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from July to December 2023, after gaining the approval of the Institutional Review Board (Certificate No. BS AHS/MIC-1/IRB/23/2354). The World Health Organization (WHO) formula for sensitivity and specificity was used to calculate the sample size, calculated to be 52, by keeping the expected sensitivity of 99.00%, expected specificity of 98.00%, confidence level of 95.00%, precision 5.00% and population proportion of syphilis patients as 29.50%.3, 10 However, a total of 55 serum samples were included in the study using non-probability purposive sampling technique.

Inclusion Criteria: Serum samples received from patients with clinical suspicion of syphilis were included in the study.

Exclusion Criteria: Hemolyzed and duplicate samples from same patient were excluded.

All 55 serum samples underwent both ELISA and TPHA. The samples were considered 'true positive' if they tested positive on both ELISA and TPHA whereas the samples which tested negative on both ELISA and TPHA were considered 'true negative'. Three samples showed discrepancy between these two treponemal tests and were excluded from the study. ICT was then performed on a total of 52 serum samples, and the results were recorded by a different person who was blinded to the results of ELISA and TPHA. All tests including TPHA, syphilis ELISA and ICT were performed as per manufacturers' instructions. Randox Syphilis (SYP TPHA) kit[11] was used for performing TPHA test where a volume of 25 µl of test was added in the labelled wells, followed by addition of 75 µl of control cells in the control wells and 75 µl of test cells in the test well after which the plates were covered, and the results were read visually after 45-60 min. A positive test was indicated by formation of a mat on the base of the well and a negative test by appearance of a button in the well. The results were interpreted as per manufacturer's instructions. TP ELISA Antibodies to Treponema pallidum (TP) ELISA Test Kit (Antec Diagnostic products, UK) was used to perform ELISA. A 50 µl of positive control, negative control and specimen were added into their respective wells except blank and afterwards, 50 µl of enzyme conjugate was added to each well except blank. Plates were incubated at 37°C for 60 minutes. After washing 5 times, 100 µl of substrate was added to each well and plates were incubated at ambient temperature in the dark for 20 minutes, then, 50 µl of stop solution was added to each well and the absorbance was read at 450 nm, within 10 minutes of adding the stop solution. The results were interpreted according to manufacturer's instructions. ICT was performed using R-test Syphilis Ab Rapid test (Lab Diagnostic Systems (SMC) pvt Ltd)[12] where one drop (30-45 µl) of serum was added to the sample well after labelling the device with the individual's identification (ID) number. This was followed by addition of one drop of diluent and result was read in 15 minutes. The test was declared positive if there was appearance of red bands in both the control (C) and test (T) areas. Appearance of a colored band in 'C' region and no band in 'T' region indicated a 'Negative' test, however, the test was declared as 'Invalid' if either No Band was seen in both 'C' and 'T' regions or only one band appeared in 'T' region and no band seen in 'C' region. The data were recorded in Microsoft Excel 2019 and analyzed using International Business Machines Corporation (IBM) Statistical Package for Social Sciences (SPSS) version 26.00. Qualitative variables were presented as frequencies and percentages. The results of ICT were compared against the true positive and true negatives detected by the reference method (ELISA/TPHA combination) using ECDC algorithm. For inferential statistics, Cohen's kappa, sensitivity, specificity and accuracy were calculated.

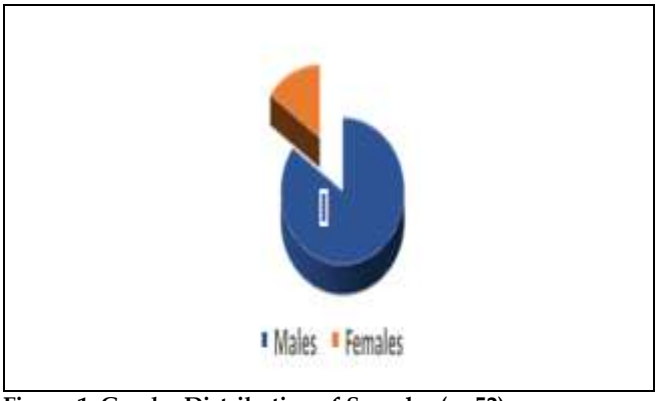

Figure-1: Gender Distribution of Samples (n=52)

RESULTS

A total of 55 serum samples were subjected to ELISA and TPHA for the diagnosis of syphilis, where 3 samples had discordance between results of TPHA and syphilis ELISA and were not included in

subsequent analysis. Out of the remaining 52 serum samples, majority were from male patients as shown in Figure-1), and 28 samples were found to be true positive and 24 were found to be true negative on concordance of both TPHA and ELISA.

The comparison of results of ICT against the combined results of TPHA and ELISA as per ECDC algorithm, using a 2x2 contingency table, as shown in Table-I, revealed that ICT had a diagnostic accuracy of 98.07%.

Table-I: Comparison of ICT (R-test Syphilis Ab Rapid test) with ECDC Algorithm (n=52)

		R-test (ICT)	
		Positive	**Negative**
Combination of TPHA/ELISA (ECDC* algorithm)	Positive	27 (52.00%)	01 (2.00%)
	Negative	00 (0.00%)	24 (46.00%)
Sensitivity = 27/(27+1) = 0.964			
Specificity = 24/(24+0) = 1.00			
Diagnostic Accuracy = (27+24)/52 =0.9807			

**ECDC: European Center for Disease Prevention and Control, ICT: Immunochromatographic test*

The percentage of agreement between the results of ICT and combined results of TPHA and ELISA was evaluated by Cohen's kappa coefficient (0.96) and the result was 98.36% and as per Landis and Kock, this agreement falls in the category of 'Almost Perfect'.[13]

DISCUSSION

The results of this study showed a satisfying level of agreement (98.36%), when ICT was compared with ECDC approach, in terms of identifying both positive and negative cases. Moreover, the sensitivity and specificity of ICT were found to be 96.43% and 100.00% respectively which are comparable to a study conducted at a tertiary care teaching hospital in North India, in RPR was used as a primary screening test of syphilis in blood donors and other lab tests for diagnosis of syphilis i.e. TPHA, ICT and ELISA were performed for comparison and noted that ICT had a sensitivity of 98.80% and specificity of 97.80%, and when TPHA and ICT were compared, a high kappa index (0.98) was obtained.[10] On the other hand, absence of any false positive result indicates a high specificity for ICT, with less chance of misclassifying syphilis negative samples as syphilis positive. A prospective study, involving 633 suspected syphilis infected patients from Burkina Faso, was done to evaluate the diagnostic performance of ICT (SD Bioline Syphilis 3.00) in comparison to VDRL and TPHA combination for detection of treponemal antibodies, where ICT had sensitivity, specificity, PPV and NPV of 100.00%, 92.00%, 85.95% and 100.00% respectively, concluding that ICT had good diagnostic performance compared to VDRL and TPHA tests.[14] A cross-sectional study of 90 volunteer blood donors, showed three false positives and one false negative on ICT, and the calculated sensitivity and specificity of ICT in comparison to ELISA was found to be 75.00% and 97.70% respectively, and suggested more advanced tests, these being ELISA and ICT.15 A study was conducted to comparatively evaluate rapid immunochromatographic test (SD Bioline Syphilis 3.00) for Treponema pallidum specific antibody and the TPPA (Serodia) assay and discrepancies between these two were sorted by FTA-ABS, over 132 samples in the study with 78 syphilis positive and 54 syphilis negative, with SD Bioline Syphilis 3.00 having percent agreement of 99.20% and kappa value of 0.98, however, one false positive sample was detected by TPPA, with the overall performance of both tests being the same with ICT being superior to that of TPPA, as ICT can be used in clinical settings due to its simplicity.[16] A comparative study conducted in Brazil, using stored sera at -20°C for evaluation of the Abbott Determine Rapid Syphilis TP assay (Dainabut Co. Ltd., Tokyo, Japan) for serodiagnosis of syphilis by comparing it with VDRL (Laborclin, Paraná, Brazil) testing and TPHA (Biolab, BioMérieux, Rio de Janeiro, Brazil). In this study, 250 TPHA positive, 17 TPHA indeterminate and 300 TPHA negative samples were included. Among 250 TPHA positive samples, 195 samples were also VDRL reactive while all TPHA negative samples and 14 indeterminate samples were VDRL non-reactive. Three people separately performed the Abbott Determine Rapid Syphilis TP assay on these samples and interpreted results, noting the Abbott Determine Rapid Syphilis TP assay having a sensitivity of 95.60 to 98.40% and specificity from 95.70% to 97.30%.[17] At CDC laboratories, a study was conducted to evaluate the rapid lateral flow immunochromatographic assay, Syphilis Health Check (SHC), (Trinity Biotech USA, Inc., Jamestown, NY) by comparing it with other treponemal tests T. pallidum passive particle agglutination (TP-PA) test (Fujirebio Diagnostics, Inc., Malvern, PA), ELISA and CLIA and non-treponemal test RPR (Arlington Scientific, Inc., Springville, UT) as the Syphilis Health Check (SHC) assay is the only Food and Drug Administration (FDA)-cleared rapid syphilis test and in this study, 1,406 stored serum samples were included, noting that rapid tests like the SHC have the

potential to enhance access to early diagnosis and treatment, particularly in resource-limited settings or among highly vulnerable people where prompt treatment is necessary to prevent complications.[18]

LIMITATIONS OF STUDY

This study had a small sample size due to limited resources, and although there is a very high agreement of ICT with ECDC algorithm based serodiagnosis of syphilis in our study, however a larger scale study with random sampling will lend further credence to these findings.

CONCLUSION

For serodiagnosis of syphilis, compared using ECDC algorithm (positivity on two treponemal tests such as TPHA/ syphilis ELISA), the treponemal ICT (R-test Syphilis Ab Rapid test) was found to be a highly sensitive, specific and accurate test.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZUD & MMG: Data acquisition, data analysis, critical review, approval of the final version to be published.

IAM & MOR: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SHN & AI: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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