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ORIGINAL ARTICLE

Is the Second Treponemal Test Necessary in the Reverse Algorithm for the Diagnosis of Syphilis?

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SUMMARY

Background: **This study was performed to investigate the necessity of the second treponemal test and to evaluate the diagnostic performance of the first treponemal test in the reverse algorithm of syphilis diagnosis.**
Methods: **Abbott Architect Syphilis TP assay, a chemiluminescence immunoassay (CIA), was used as the first step treponemal test. Treponema pallidum haemagglutination assay (TPHA) test results of reactive samples from the first test were recorded. TPHA test result was considered as confirmatory. TPHA test results were grouped according to their Abbott Architect Syphilis TP assay results and they were compared with Mann-Whitney *U* test. For Abbott Architect Syphilis TP assay, a cutoff value with 100% specificity was determined via a ROC curve analysis which would render TPHA test unnecessary.**
Results: **Out of 146,800 samples 2,646 were reactive in the first step. Of those, 2,002 had a TPHA test result. Of the 2,002 TPHA tests, 1,706 were positive and 296 were negative. TPHA positive and negative groups have significantly different CIA signal/cutoff values. Using a ROC curve built for evaluation of the first-step test, the maximum Youden's index value was found as 5.26. If this value would be accepted as cutoff, it would have a specificity of 85%. The specificity of 100% can be reached if a new cutoff value is set to 27.83.**
Conclusions: **Calculated cutoff value with 100% specificity is not practically applicable. It achieves saving of TPHA test in only six percent of reactive samples. Architect Syphilis TP assay is advantageous in large laboratories but is not enough to lead diagnosis without a second treponemal assay. Therefore it was decided to continue the reverse algorithm with dual treponemal assays.**
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INTRODUCTION

Though it is treatable, syphilis due to *Treponema pallidum subsp. pallidum* is a disease observed all over the world. It can not be cultured in artificial media *in vitro*. Serological methods are an important part of the diagnosis of the infection. At the present time, there are three main algorithms using serological testing methods: traditional algorithm which begins with nontreponemal tests, two different reverse algorithms suggested by CDC, and ECDC which begin with treponemal tests. Use of reverse algorithm has increased with time as en-

zyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays (CIA) in automated systems have become more available [1]. These systems allow a large number of samples to be processed in a short time. Thanks to these capabilities, laboratories serving large populations can begin their diagnostic algorithms with treponemal tests.

The aim of our study was to investigate the necessity of the second treponemal test. In order to give results in a shorter time and save on the costs of second treponemal test, we analyzed the performance characteristics of the first treponemal test.

MATERIALS AND METHODS

Samples and tests

Serum samples sent to our laboratory between May 2014 and December 2018 were included in the study. Abbott Architect Syphilis TP (Abbott, Germany) assay was used as the first treponemal test. This assay is a chemiluminescent microparticle immunological assay which uses recombinant antigens belonging to *Treponema pallidum* to detect specific antibodies. Signal/cutoff values which are equal or higher than one are considered as reactive. Signal/cutoff values belonging to reactive samples were recorded.

TPHA (Treponema pallidum haemagglutination assay, Plasmatec, UK) test results of samples whose first treponemal test results were reactive were recorded. TPHA assay uses avian erythrocytes coated with antigens of *Treponema pallidum* Nichols strain. Specific antibodies in the sample cause agglutination which is interpreted as positive. TPHA test was considered as the confirmatory test. Samples are divided into two groups as TPHA positive and negative.

Statistics

Median and quartile values were calculated for non-normally distributed signal/cutoff reactive results from Abbott Architect Syphilis TP assay. Distribution of signal/cutoff values of TPHA positive and negative samples was compared with the Mann-Whitney *U* test. The relationship between signal/cutoff values from the Architect Syphilis TP assay and confirmatory TPHA test results was investigated with receiver operating characteristic curve analysis. Axes of the curve were composed of sensitivity and false positivity values. The Youden's maximum index was used to calculate the best sensitivity/specificity combination. A cutoff value with 100% specificity was determined which would render the TPHA test unnecessary.

RESULTS

Between the before mentioned dates, 2,646 samples out of 146,800 samples gave reactive results with Abbott Architect Syphilis TP assay. Six hundred and forty-four of them were lacking TPHA results and therefore excluded from the study. Signal/cutoff results of the remaining 2,002 samples can be seen in Figure 1. Positively skewed data of signal/cutoff values had a median of 12.02, first quartile of 5.97, and third quartile of 17.095.

Out of 2,002 samples, 1,706 had positive TPHA test result. Samples with positive TPHA test result had a median value of 12.99, first quartile value of 8.45, and third quartile value of 17.90. Two hundred and ninty-six samples with negative TPHA test results had a median value 2.08, first quartile value of 1.46, and third quartile value of 3.48 (Figure 2). Signal/cutoff values of TPHA positive and negative results were significantly different from each other ($p < 0.001$, Mann Whitney *U* Test). ROC curve was prepared with Abbott Architect Syphilis TP assay results accepting TPHA results as confirmatory (Figure 3). Using Youden's maximum index, optimal signal/cutoff value was calculated as 5.26. At this value, specificity was 85%. Required signal/cutoff value to achieve 100% specificity was 27.83. Only 6 % of all reactive Abbott Architect Syphilis TP assay results were above this value.

DISCUSSION

A serological test used at the diagnosis of syphilis provides a presumptive diagnosis [2]. All test results must be evaluated with clinical findings and patients' medical history. This study aimed to find a cutoff value for the first step CIA test, Abbott Architect Syphilis TP assay in this case, which would render second step confirmatory TPHA test unnecessary and therefore would be enough to diagnose syphilis accurately. In this way, it would be possible to reduce the cost of syphilis diagnosis and to decrease the turnaround time for final test results. This topic has previously been investigated in different studies in literature. Details about these studies can be seen in Table 1.

A cutoff value of 27.83 provided 100% specificity. This high cutoff value can be attributed to a large number of samples. Suggested cutoff values from different studies increase as the number of samples increase as can be seen in Table 1. Another reason can be prevalence. Although the exact prevalence of syphilis is unknown in Turkey it is estimated low [11]. Prevalence in our study was found as 1.8%. It is known that the reverse algorithm can result in false positivity in populations with a low prevalence [8,12-15]. For this reason, some samples with high signal/cutoff value had exhibited negative TPHA test results.

A major limitation of this study was that it lacks clinical data which is essential for definitive syphilis diagnosis. In order to evaluate performances of tests, there is a need for studies involving both laboratories and clinics. Another limitation may be the use of TPHA test as a confirmatory second treponemal test. Though European guideline allows usage of the TPHA test [2], the most

Table 1. Studies investigating cutoff values for the first treponemal test.

Study	1st Treponemal assay	Sample number	Cutoff value	Specificity-
Özbek A. et al. [3]	CIA	92	12	100
Kyunghoon Lee et al. [4]	CIA	126	9	100
Yi J. et al. [5]	EIA	139	21.45	95
Jonckheere S. et al. [6]	CIA	178	5.6	97.3
Lee S. et al. [7]	ECLIA	212	3.6	91.7
Dai S. et al. [8]	CIA	319	9.9	100
Serhir B. et al. [9]	CIA	668	16.4	100
Zhuang Y. H. et al. [10]	CIA	2,259	50.0	100

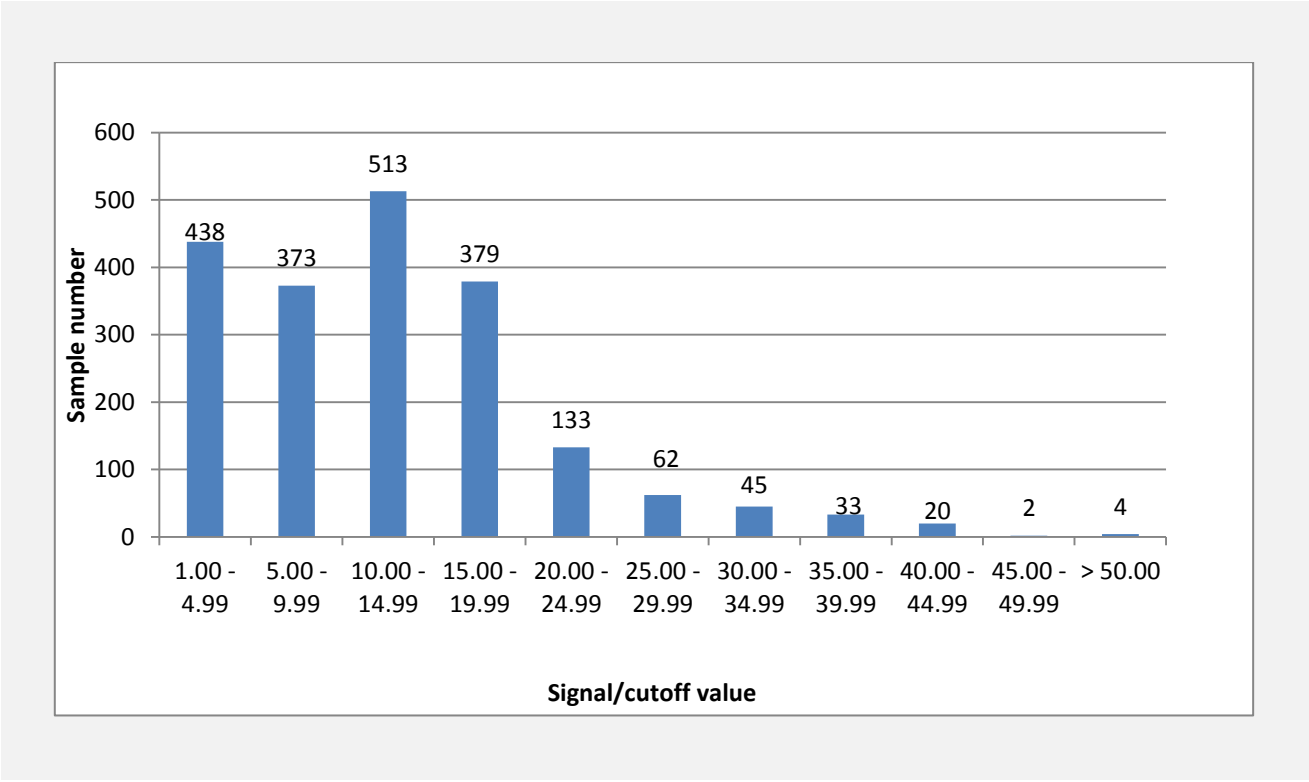

Figure 1. Signal/cutoff value distribution and sample number.

widely accepted second treponemal test is *Treponema pallidum* particle agglutination assay (TPPA) [8,16]. As TPHA test is commonly used in our country, generalization of acquired results should not be a problem [3, 11].

CONCLUSION

Calculated cutoff value with 100% specificity is not practically applicable. It achieves saving of TPHA test in only six percent of reactive samples. Architect Syphilis TP assay is advantageous in laboratories with large sample volumes but its positive predictive value in low prevalence populations is not enough to lead diagnosis without a second treponemal assay. Therefore, it was decided to continue the reverse algorithm with dual treponemal assays.

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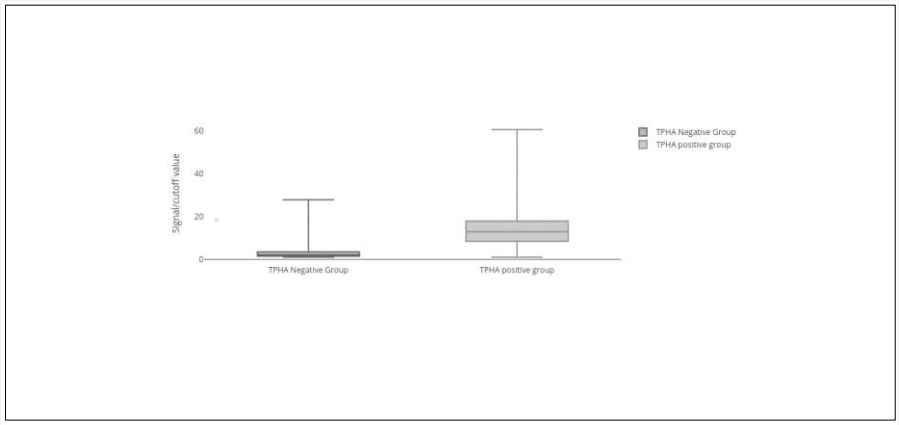

Figure 2. Distribution of signal/cutoff values of TPHA positive and negative groups.

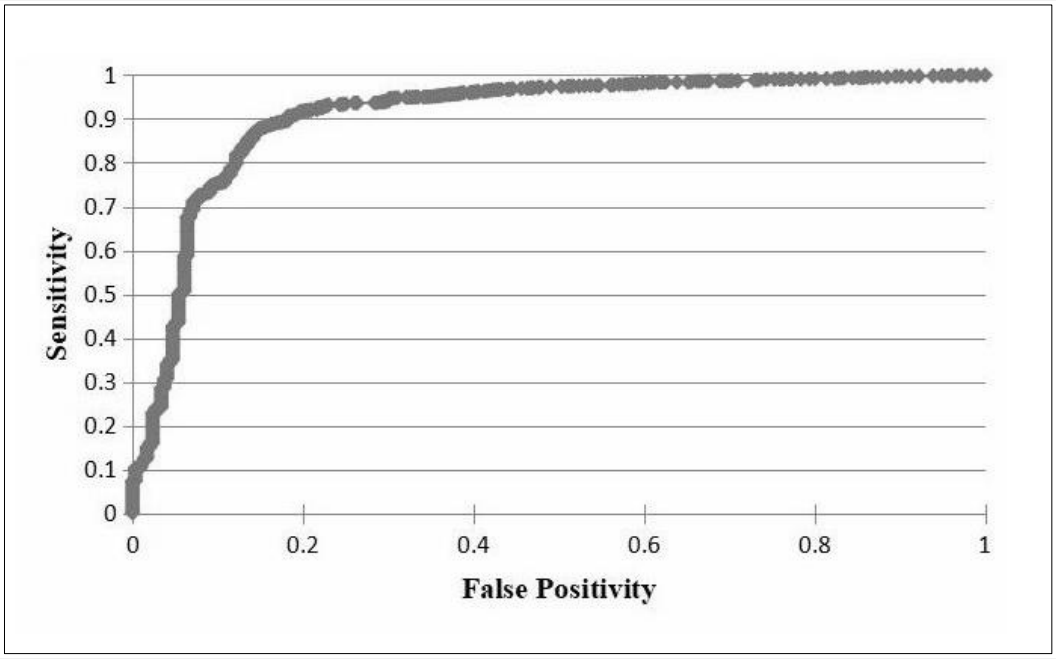

Figure 3. ROC curve analysis of signal/cutoff values and confirmatory TPHA test results.

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Both authors contributed according to the ICMJE recommendations.

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Declaration of Interest:
None.

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