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Serological Diagnosis of Syphilis: Preliminary Study Searching for an Algorhitm in Turkey

Cemile Sonmez, Tulin Demir, Tahir Uzunmehmetoglu, Hakan Maden, Selcuk Kilic

Public Health Institution of Turkey, Microbiology Reference Laboratories, Ankara, Turkey

Corresponding author:
Cemile Sonmez, MD
Public Health Institution of Turkey
Microbiology Reference Laboratories
Adnan Saygun Street no:55 Sihhiye
Ankara
Turkey
cemilesonmez2004@yahoo.com

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ABSTRACT Different algorithms have been proposed to increase the diagnostic capacity of syphilis. We analyzed three common algorithms for detecting suspected syphilis cases in low prevelance Turkish population. The study included a total of 340 clinical serum samples from adults throughout Turkey, who had syphilis as a clinical preliminary diagnosis and were positive on at least one of the following tests: Rapid Plasma Reagin (RPR), *Treponema pallidum* Haemagglutination test (TPHA) and FTA-abs Ig. In adittion to percent agreement, kappa coefficients were calculated to compare the conformity between the three algorithms. Both the reverse and the ECDC algorithms had higher diagnostic efficacy than the conventional algorithm. The sensitivity/specificity/ accuracy of conventional, reverse and ECDC algorithms were 51.3%/86.1%/55%; 80.9%/86.1%/81.4% and 80.9%/100%/82.9% respectively. The interrater reliability was moderate for conventional-reverse algorithm (73.53%; κ=0.484; 95%CI=0.41-0.56; p=0.001) and conventional-ECDC algorithm (72.06%; κ=0.454; 95% CI= 0.37-0.54; p=0.001), and near perfect for reverse-ECDC algorithm (98.53%; κ=0.963; 95% CI=0.93-0.99; p=0.0001). Our data support the use of ECDC algorithm in serological diagnosis of syphilis. It may increase the diagnostic capacity if treponemal tests are used for screening, and then positive results are confirmed with a different and second treponemal test.

KEY WORDS: *Treponema pallidum*, syphilis, serology, algorithm, sexually transmitted infections

INTRODUCTION

Syphilis caused by a spirochet *Treponema pallidum* subsp. *pallidum* has been an important public health problem for many years all over the world (1). The inability of culturing the bacteria easily has forced laboratorians to search for alternative methods for diagnosing syphilis (2). Direct diagnosis is based on the examination of specimens obtained from genital ulcers or dermatologic lesions by dark field microscopy. Although the dark field microscopy is a reliable diagnostic tool, the accuracy of the test is affected by the experience of the person performing the test, the number of live treponemas in the lesion and the presence of nonpathogenic treponemes in the lesions (3). While recent advances in molecular methods, such as PCR, look promising, the test largely remains a research tool as it is still not available in many diagnostic laboratories (4).

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Serological tests for the detection of nontreponemal antibodies or antibodies against *Treponema pallidum* in all stages of infection, remain the mainstay of diagnosis. Non-treponemal tests are largely used to monitor the status of infection, while treponemal tests are primarily used to confirm the presence of infection. The sensitivity and specificity of treponemal and nontreponemal tests vary with the type of test as well as the stage of infection. Antibodies detected by treponemal tests arise earlier than those detected by non-treponemal tests and typically remain detectable for life, even after successful treatment (4-5).

There are currently two commonly used approaches for the serological diagnosis of syphilis: the conventional algorithm and the reverse algorithm. In the conventional algorithm, a non-treponemal test, such as the Venereal Disease Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR) test, with positive results is confirmed using a specific treponemal test such as TPHA or FTA-abs (6,7,8). However non-treponemal test based screening may not always be followed by a treponamal test, especially in resource-limited settings, and may therefore miss previously treated, early untreated, and late latent cases (9,10). Therefore, positive non-treponemal test results should be confirmed with treponemal tests, such as Fluorescent Treponemal Antibody-absorption (FTA-abs) test, *Treponema pallidum* Hemagglutination test (TPHA) or ELISA tests that detect specific antibodies against *T. pallidum* proteins (6,7,11,12).

The use of a single serologic test is often insufficient in the determination of false negative or positive test results. For this reason, different algorithms have been proposed to increase the diagnostic capacity. In the serological diagnosis of syphilis, two algorithms are defined as conventional (conventional-CDC) and reverse (reverse and ECDC) (13). The conventional algorithm is the most widely accepted method worldwide, which is recommended by the CDC, and involves confirmation of positive non-treponemal tests with one of the treponemal tests (8,10,11,14-17). In recent years, the reverse algorithm, in which two implementation schemes are defined, is the alternative algorithm proposed for conventional approach. In the first scheme of reverse algorithm, treponemal tests are first performed as a screening test and the positive results obtained are confirmed by non-treponemal tests. When the non-treponemal test result is negative, a second, different treponemal test is used (11). In the second scheme which is proposed by the ECDC, non-treponemal test is not used, and the results that are positive by screening with the treponemal test are confirmed by a second different treponemal test (1,18-20).

In this study, we aimed to evaluate the compatibility of the three different algorithms (conventional, reverse and ECDC) which are used in syphilis diagnosis nowadays.

METHODS

Syphilis diagnostic tests are conducted in Public Health Institution of Turkey, Sexually Transmitted Diseases Laboratory which is accredited for syphilis tests. Patient sera with syphilis as a clinical preliminary diagnosis, which were tested routinely and stored at -80° C between 2013-2015 years were evaluated comparatively. A total of 340 serum samples positive by either RPR, (Omega, UK), TPHA, (Plasmatec, UK) and FTA-abs IgG/IgM (Euroimmun, Germany) tests were included in the study. All the serological testing was performed on the same specimen according to the manufacturers' recommendations, and the results of all the tests were reported simultaneously. Conventional, reverse and ECDC algorithms were evaluated for compatibility with each other.

***Conventional algorithm*:** The nontreponemal test (RPR test) was used as a screening test. The positive non-treponemal test results were confirmed by one of the treponemal tests (TPHA test).

***Reverse algorithm*:** In the first stage, treponemal test (TPHA) was used and positive results were confirmed by non-treponemal test (RPR test). A second different treponemal test (FTA-abs test) was applied to the samples detected negative by the non-treponemal test.

***ECDC algorithm*:** After screening with treponemal test (TPHA test), positive results were confirmed by a second different treponemal test (FTA-abs).

Statistical analysis: The diagnostic accuracy of the tests was assessed using the SPSS 15.0 program (IBM, USA). Percent agreement and κ (kappa) coefficient analysis were used to determine the correspondence between the algorithms. The percentage of agreement between the results according to κ values were categorized as near perfect (0.81-1.0), substantial (0.61-0.8), moderate (0.41-0.6), fair (0.21-0.4), slight (0-0.2), or poor (<0) (21).

RESULTS

Of 340 serum samples tested, 198 (58.2%), 262 (77%), 304 (89.4%) were positive by RPR, TPHA and FTA-abs respectively. 161 (81.3%) of RPR-positive samples and 101 (71.1%) of RPR-negative samples were positive on TPHA test. RPR-positive and TPHA-negative 37 (10.8%) samples were considered as biological false positive reaction (Figure 1). According to

Table 1. Comparison of diagnostic capacities of algorithms

Algorithm		FTA-abs (Gold standard)		Sensitivity	Specificity	PPV	NPV	Accuracy	95% CI
		+	_						
Conventional	+	156	5	51.3	86.1	96.8	17.3	55.0	0.076-0.197
	-	148	31						
Reverse	+	246	5	80.9	86.1	98	34.8	81.4	0.296-0.517
	-	58	31						
ECDC	+	246	0	80.9	100	100	38.2	82.9	0.368-0.578
	-	58	36						

FTA-abs, Fluorescent Treponemal Antibody Absorption test; PPV, Positive Predictive Value; NPD, Negative Predictive Value; ECDC, European Centre for Disease Prevention and Control; 95%CI, 95% confidence interval

the gold standard FTA-abs test as the gold standart method, sensitivity, specificity and accuracy of RPR and TPHA tests were; 56.9%, 30.5%, 54.1%; 80.9, 55.5%, 78.2%, respectively. RPR test results showed slight agreement with TPHA (59.4%; κ=0.109; 95%CI=0.01-0.20; p=0.08) and TPHA showed fair agreement with FTA-abs test results (78.24%; κ=0.241; 95%CI=0.12-0.36; p=0.001). The interrater reliability was moderate for conventional-reverse algorithm (73.53%; κ=0.484; 95%CI=0.41-0.56; p=0.001) and conventional-ECDC algorithm (77.06%; κ=0.454; 95% CI=0.37-0.54; p=0.001) and near perfect for reverse-ECDC algorithm (98.53%; κ=0.963; 95% CI=0.93-0.99; p=0.0001).

Of the 304 samples detected positively by FTA-abs, 161 (52.9%) were positive with the conventional algorithm, 251 (82.5%) with the reverse algorithm and 246 (80.9%) with the ECDC algorithm. Furthermore, the sensitivity of the conventional testing algorithm was only 51.3%, with the lowest negative predictive value of 17.3%. The sensitivity of reverse and ECDC algorithm was found to be 80.9% and the specificity was found to be the highest in the ECDC algorithm (100%). The algorithms' specificity, positive predictive value, negative predictive value and accuracy are presented in Table 1. When conventional, reverse and ECDC algorithms were evaluated for false negativity and positivity, false negativity was 48.7%, 19.1%, and 19.1%, respectively. False positivity was 13.8%, 13.8% and 0%, respectively. Positive test result probability ratios were 3.6%, 5.8% and 80.9% according to conventional, reverse and ECDC tests, respectively. Negative test result probability ratios were 56.5%, 22.1% and 0.19%, respectively. Both the reverse and ECDC algorithm had high accuracy in the diagnosis of syphilis. However, the ECDC algorithm is slightly different from the reverse algorithm as it does not involve a nontreponemal test for diagnosis. Therefore, we compared these 2 testing algorithms using κ coefficient analysis. The result indicated a high degree of consistency between the reverse and the ECDC algorithms (Figure 1 and Table 1).

DISCUSSION AND CONCLUSION

The diagnosis of syphilis is challenging and is generally made by serologic tests (12). Currently, there are a few studies that evaluate different algorithms in detail in terms of syphilis. Although the findings in our study are critical in assessing the performance of the algorithms, an inherent limitation of the study was the lack of detailed clinical information on the patient's symptoms/signs or stage of the disease. Because of this limitation, we were unable to evaluate the diagnostic accuracy of the algorithms compared with clinical diagnosis. As the incidence of the disease is not known exactly in Turkey, we sought to directly compare the algorithms in a patient population with a low prevalence of syphilis.

In our study, 58.2% of the total 340 sera detected positively by any test were found to be positive with RPR, 262 (77.05%) with TPHA and 304 (89.41%) with FTA-abs. RPR and TPHA are widely used in our country and there is a need for a standard algorithm for the serological diagnosis of syphilis. 71.1% of the RPR-negative samples were detected as positive with TPHA and were defined as false-negatives. Non-treponemal tests that detect antibodies against cardiolipin are not specific to treponemal infections. In our study, 37 (10.8%) of all the samples positive with a RPR test were negative with TPHA test and were defined as false-positives. False positive non-treponemal test results may be seen in many conditions such as HIV, SLE, tuberculosis, rickettsial infection, spirochetal infections other than syphilis, bacterial endocarditis, autoimmune diseases, vaccination, pregnancy, intravenous drug use and advanced age not related to syphilis (9,10).

The most commonly used approach in diagnosis is based on screening with non-treponemal tests and confirmaton of positive results with treponemal tests

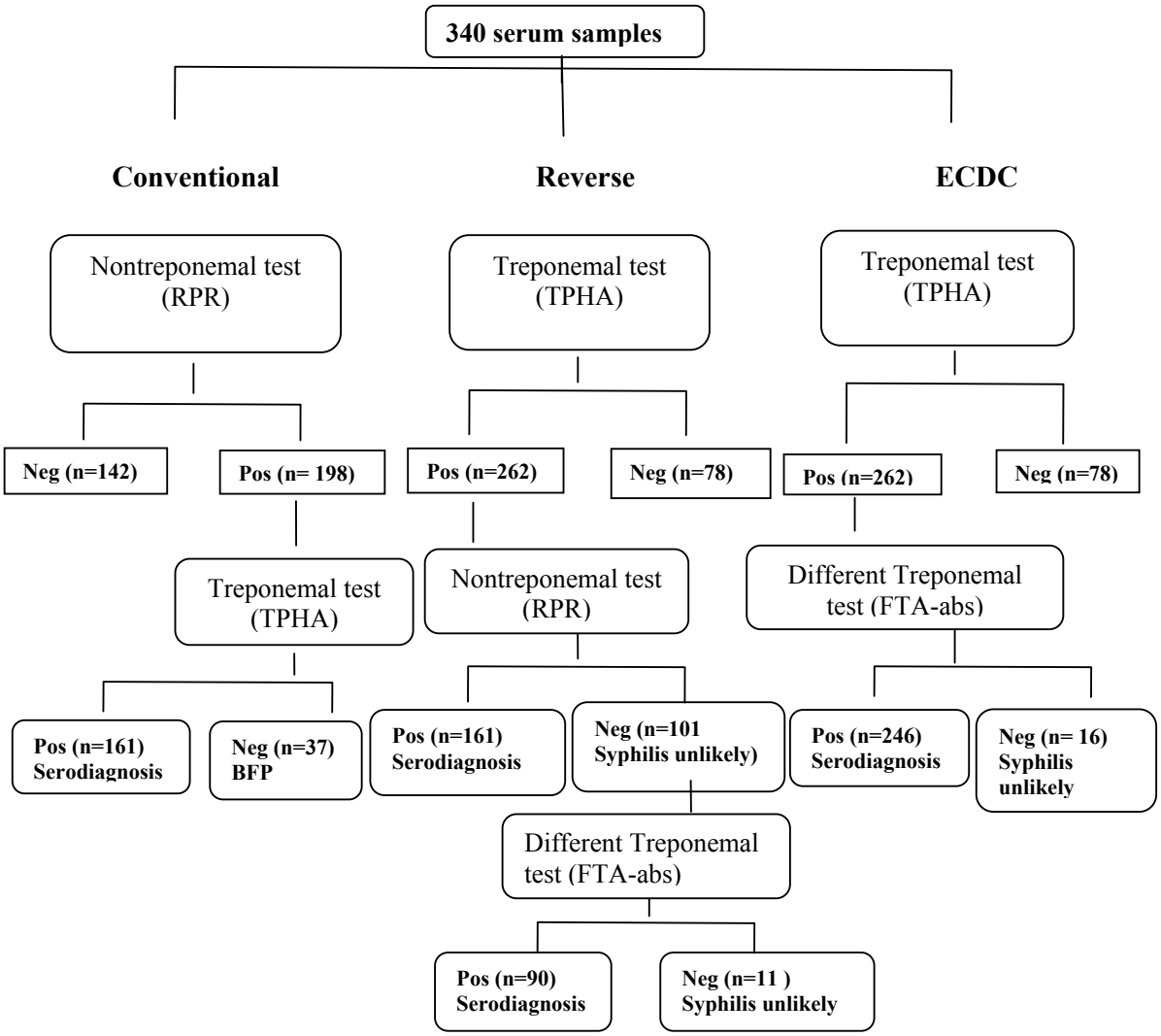

Figure 1. Different testing algorithms for syphilis serological diagnosis. Abbreviations: ECDC, European Centre for Disease Prevention and Control; RPR, Rapid Plasma Reagin; TPHA, *Treponema pallidum* Hemagglutination Assay; FTA-abs, Fluorescent Treponemal Antibody Absorbtion Test; BFP, biological false positive

as in the conventional algorithm (11). This approach is based on studies showing that RPR screening is more compatible with disease activity than the reverse algorithm (11,22). In our study, 52.9% of the cases were defined as syphilis with the conventional algorithm. In the conventional algorithm, negative non-treponemal test results are not evaluated by treponemal tests. Since 71.1% of the RPR-negative patients were positive with the treponemal test, it is thought that the conventional algorithm was insufficient in syphilis diagnosis. Tests that detect specific antibodies against treponemal antigens have a higher sensitivity than non-treponemal tests so this issue changed the conventional approach (6). However, as treponemal tests are more difficult and expensive, their use as a screening test is limited. Furthermore, unlike non-treponemal tests, which usually become negative with effective treatment, treponemal tests usually remain life-long positive. Hence, treponemal tests are not used in the follow-up of treatment efficacy as are non-treponemal tests (23). The US Center for Disease Control (CDC) reported that the percentage of patients with false-positive treponemal screening in low-prevalence populations was especially high as it was 2.9-times greater than those in high prevelance populations (24). Accordingly, the implementation of the reverse algorithm has created a substantial amount of confusion and concern among healthcare providers and patients. Therefore, the CDC continues to recommend the conventional algorithm (24).

In line with the results obtained from studies (1,18,25), most laboratories apply the reverse algorithm, which includes a non-treponemal test. When screening with the treponemal test, a larger number

of positive test results can be obtained than with the conventional algorithm, but a significant number of them can be found to be negative with RPR test, depending on stage and treatment status (20,26). In our study, the screening for syphilis using a treponemal test detected a higher number of patients with reactive results compared to conventional screening. Since 1982, WHO recommends screening and diagnosis of syphilis by non-treponemal and treponemal tests and European countries propose screening with treponemal tests (17). Binnicker *et al.* (12) reported that the reverse algorithm resulted in higher false-positive results than the conventional algorithm, and the false positive rates for reverse and conventional algorithms were 0.6% and 0% respectively. Similarly, in the study of CDC (11), the false positive rate with the reverse algorithm was determined to be 0.6%. In our study, the false positive rates for conventional, reverse and ECDC algorithms were determined as 13.8%, 13.8% and 0%, respectively, and higher false positivities were observed with reverse algorithm according to other studies (11,12). Despite our findings, further research is required to understand the real cause of false positivity. Similar to Binnicker *et al* (12) recommendation, we consider that the diagnostic capacity of reverse algorithm is higher than the conventional method, and for this reason we emphasize that it is appropriate to use it in the diagnosis. In adittion, the results we have obtained show that in reverse algorithm ECDC scheme has high sensitivity, specificity and accuracy, and because of the low false positivity, the substitution of the conventional method may result with increasing sensitivity in diagnosis.

The screening stategy recommended by the ECDC involves a primary treponemal screening test followed by a second confirmatory treponemal test (20,27). The results obtained from a large cohort (1), as well as our data support performing ECDC algorithm for syphilis screening in Turkish population also. The direct comparison of the revers and ECDC algorithm in our study gave an overall percent agreement and kappa value of 98.53% and 0.963, suggesting that a nontreponemal test is unnecessary for syphilis serodiagnosis. When the diagnostic capacity of both tests is compared with the conventional algorithm, the use of reverse and ECDC algorithms is proposed. Similar to the data obtained from our study, it has been reported by Tong *et al* (1) that reverse and the ECDC algorithms have a higher diagnostic capacity than the conventional algorithm. We also found almost perfect agreement (κ=0.996) between reverse and ECDC algorithms. This finding was similar to our study (κ = 0.963). However, as Simcic *et al* (28) reported, we also thought that the use of a treponemal test for screening purposes and confirmation of the positive results with a second (ECDC algorithm) will increase sensitivity and specificity. In the cases that the first treponemal test is positive and the confirmatory treponemal test is negative, then it is not clear whether the first screening test is a false positive or is more sensitive. This is a disadvantage of the algorithm, so it would be advisable for a laboratory to select two treponemal assays with comparable performance to avoid discrepant results (27).

There are limited studies on the comparison of syphilis diagnostic algorithms in Turkey. In the study conducted by Ozbek *et al* (6), serum samples were examined by an ELISA test using treponemal antigens. According to the results of the study, it was suggested that false-positive results could be obtained by ELISA test. Similar to our study, it has been concluded that the results of positive treponemal test must be confirmed by another treponemal test and RPR test should be used in order to determine the activity of the infection and for the treatment follow-up (6). There is also a need for the evaluation of ELISA tests since they are frequently used as screening tests in order to obtain an appropriate algorithm for the population.

The most important limiting factor in this study was the fact that there are very limited data on syphilis prevalence in our country. The actual incidence of syphilis is not known because of differences in the algorithms used, inappropriate test selection, and empirical treatment without confirmation of positive test results. Additionally, inappropriate patient selection and lack of nationwide testing strategy playes a role in ambigious estimation of incidence. For these reasons, a laboratory-based surveillance network should be established throughout the country and standardization of the tests used in syphilis serology should be one of the main targets. Syphilis diagnostic algorithms applied in different countries vary significantly depending on the considerations (29). Given the pros and cons of each diagnostic algorithm, the decision of using a treponemal or nontreponemal test as the first screening test should be based on a combination of factors: local syphilis prevelance, the expected workload and the available budget for labor and consumables.

In conclusion, we suggested that non-treponemal tests should be replaced by treponemal tests in syphilis diagnosis as a first screening test. This requires new diagnostic algorithms to be established. According to our results, using treponemal test for screening purposes would increase the diagnostic sensitivity and specificity. Positive results should also

be confirmed with a different second treponemal test (ECDC algorithm). Once syphilis has been diagnosed, nontreponemal test can be performed to assess disease activity and follow treatment status.

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