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Evaluation of RPR, TPHA and ELISA test results used in the diagnosis of syphilis at a university hospital

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ABSTRACT

Aims: This study aimed to evaluate the laboratory results of treponemal and non-treponemal serological tests used in the diagnosis of syphilis and to investigate the diagnostic contributions of different testing algorithms.

Methods: Between April 2023 and June 2025, serum samples from 600 patients who underwent all three tests in the microbiology laboratory were retrospectively analyzed. The rapid plasma reagin (RPR), *Treponema pallidum* hemagglutination assay (TPHA) and enzyme-linked immunosorbent assay (ELISA) tests were performed according to the manufacturers' instructions, and the results were evaluated within both conventional and reverse algorithm frameworks.

Results: In 89.5% of cases (n=537), all three tests were negative, while at least one test was positive in 10.5% (n=63). ELISA showed the highest positivity rate, with 63 reactive samples. A total of 20 cases were positive across all three tests simultaneously. Samples positive only by ELISA were notably increased in the age group over 60 years. RPR positivity was lower compared to treponemal tests. Within the reverse algorithm, discrepancies between ELISA-positive and RPR-negative results were observed at a notable frequency.

Conclusion: Using treponemal tests as the first step in syphilis screening provides a more sensitive approach. Positive results should be confirmed with a second treponemal test, while non-treponemal tests are essential for assessing active infection and treatment monitoring. Higher ELISA positivity in older individuals suggests more false positives. The study underscores the need for laboratory–clinical collaboration and larger prospective studies.

Keywords: Syphilis, serologic tests, algorithms

INTRODUCTION

Syphilis is a treatable infection caused by *Treponema pallidum*, transmitted sexually, and capable of multisystem involvement.[1] It is also referred to as "the French disease" due to its emergence among French soldiers.[2] Serological tests form the basis of laboratory diagnosis for syphilis. During infection, two types of antibodies are produced: treponemal and nontreponemal antibodies. Based on the antigens used, serological tests are classified as nontreponemal tests (rapid plasma reagin (RPR), venereal disease research laboratory (VDRL)) and treponemal tests (*Treponema pallidum* hemagglutination assay (TPHA), enzyme-linked immunosorbent assay (ELISA), and fluorescent treponemal antibody–absorption (FTA-ABS)).[3] The combined use of these tests provides a comprehensive approach for syphilis screening and diagnosis.[4] Diagnostic algorithms can be examined in three main groups: In the conventional algorithm, a nontreponemal test (RPR) is performed first, and positive samples are confirmed with a treponemal test. In the reverse algorithm, the first step is a treponemal test (ELISA/TPHA), and positive samples are confirmed with a nontreponemal test. The European Centre for Disease Prevention and Control (ECDC) recommendation involves using a positive treponemal screening test followed by a different second treponemal confirmation test.[5] Since treponemal tests can remain positive for life, they are not useful for treatment monitoring. In contrast, nontreponemal tests are more suitable for treatment follow-up due to the possibility of monitoring antibody titers.[6] The tests used in syphilis diagnosis have their own specific advantages and limitations; therefore, multiple tests are generally performed together. The validity of tests used in syphilis serology may vary depending on the applied treatment and disease stage. Thus, collaboration between clinicians and laboratories is crucial for accurate diagnosis. FTA-ABS test is a highly specific

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confirmatory assay that detects antibodies against *Treponema pallidum* using fluorescent-labeled anti-human antibodies after absorption of nonspecific antibodies. It is widely accepted as the gold standard confirmatory test for syphilis and is particularly useful for confirming treponemal test reactivity in cases with discordant results.[7] This study aimed to evaluate the results of treponemal and nontreponemal tests used in syphilis diagnosis and to investigate the diagnostic contributions of different diagnostic algorithms.

METHODS

This study was approved by the Ondokuz Mayıs University Ethics Committee for Clinical Researches (Date: 15.10.2025, Decision No: 2025/492). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective study was conducted using samples sent to the faculty of medicine serology/ELISA laboratory between April 2023 and June 2025. Ethical approval for the study was obtained under. Serum samples sent to the laboratory for preoperative screening, blood donor screening, premarital screening, and clinical suspicion were retrospectively evaluated. In our laboratory, the RPR (Monlab, Spain) is routinely used as the nontreponemal test, while TPHA (Monlab, Spain) and ELISA (Cobas 801, Syphilis TP; Roche Diagnostics, Germany) are employed as treponemal tests. Only cases in which all three tests were requested simultaneously were included in the study. A total of 600 patients were evaluated, and only the first serum sample from each patient was analyzed. The collected data were retrospectively analyzed according to both conventional and reverse algorithm approaches. In the conventional syphilis screening algorithm, a nontreponemal test such as the RPR test is used as the initial screening method, and reactive samples are subsequently confirmed with a treponemal test. In the reverse screening algorithm, serum samples are first screened using an automated treponemal test, such as ELISA. Reactive samples identified by ELISA are then tested with RPR to evaluate disease activity, treatment status, and to aid in determining the stage of infection.[8] Patient blood samples were initially centrifuged at 4000 rpm for 10 minutes, and subsequent testing was performed in accordance with the manufacturers' instructions.

RPR test: Fifty microliters (50 µL) of patient serum were placed in the circles on the card test, followed by the addition of 20 µL RPR reagent. The mixture was incubated on a rotator at 140 rpm for 8 minutes. The test was interpreted as positive in the presence of clumping (agglutination) and negative if a homogeneous appearance was observed.

TPHA test: Three microplate wells were prepared for each patient. In the first well, 190 µL TPHA diluent and 10 µL serum were added to prepare a 1/20 initial dilution, from which 25 µL was distributed into the other control and test wells. Subsequently, 75 µL of the control and test suspensions were added, and the plates were incubated at room temperature for 1 hour. Results were evaluated macroscopically: sediment formation at the bottom of the well was considered negative, whereas a homogeneous appearance indicated a positive reaction. Positivity at dilutions of 1/80 or higher was considered significant.

ELISA test: ELISA tests were performed on the Cobas 801 analyzer following the manufacturer's procedures. Results were interpreted according to the manufacturer's recommendations, with a COI ≥1.0 considered reactive (positive).

RESULTS

The results of 600 patients tested at the Serology/ELISA Laboratory of Ondokuz Mayıs University Faculty of Medicine were retrospectively evaluated. Among the included cases, 279 (46.5%) were female and 321 (53.5%) were male. The mean age of the patients was 43.07 years. All three tests (RPR, TPHA, and ELISA) were negative in 537 samples (89.5%), while at least one test was positive in 63 samples (10.5%). The positive/negative distribution of the three tests is presented in Table 1. The comparative distribution of test results by year is shown in Table 2. Accordingly, the number of cases in which all three tests were simultaneously positive was 7 in 2023, 9 in 2024, and 4 in 2025. Notably, the number of samples in which ELISA was positive alone remained relatively high across all three years. Analysis of age distribution revealed that the majority of test requests were for patients aged 19–30 years. Comparative test results by age group are presented in Table 3. The cases in which all three tests were simultaneously positive were most frequently observed in the 19–40 age group, whereas cases in which ELISA was positive alone showed a marked increase in the 60 years and older age group.

Table 1. Number of patients testing positive by serological method

Result	RPR	TPHA	ELISA
Pozitive	21	39	63
Negative	579	561	537

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

Table 2. Distribution of comparative test results by year

Year	RPR+ TPHA+ ELISA+	RPR- TPHA+ ELISA+	RPR+ TPHA- ELISA-	RPR- TPHA+ ELISA-	RPR+ TPHA- ELISA+	RPR- TPHA- ELISA+	RPR+ TPHA+ ELISA-	RPR- TPHA- ELISA-
2023	7	3	0	0	0	6	0	240
2024	9	16	0	0	1	15	0	267
2025	4	0	0	0	0	2	0	30
Number of patients	20	19	0	0	1	23	0	537

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

Table 3. Distribution of comparative test results by age

Age	RPR+ TPHA+ ELISA+	RPR- TPHA+ ELISA+	RPR+ TPHA- ELISA-	RPR- TPHA+ ELISA-	RPR+ TPHA- ELISA+	RPR- TPHA- ELISA+	RPR+ TPHA+ ELISA-	RPR- TPHA- ELISA-
0-18	1	2	0	0	0	2	0	32
19-30	5	2	0	0	0	2	0	132
31-40	5	0	0	0	0	0	0	105
41-50	4	9	0	0	0	2	0	88
51-60	1	3	0	0	1	2	0	81
60<	4	3	0	0	0	15	0	99
Number of patients	20	19	0	0	1	23	0	537

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

DISCUSSION

An increase in the incidence of syphilis infections has been reported in many countries worldwide.[9] Syphilis, which affects both men and women, is frequently observed in sexually active age groups, individuals with suspected sexual exposure, homosexual men, and populations with lower socioeconomic status. Major transmission routes include sexual contact, transplacental transfer, and blood transfusion.[10] The reverse algorithm offers several advantages, including early detection of cases and the ability to avoid missing past syphilis infections that may not be detected by nontreponemal tests. However, reverse algorithm results can cause confusion and concern among patients and laboratory personnel, particularly when ELISA and RPR results are discordant (e.g., ELISA reactive, RPR nonreactive). Such results typically reflect successfully treated past syphilis cases but may also appear in non-syphilis conditions (e.g., false-reactive ELISA) or in early, late, or latent syphilis where RPR sensitivity is low.[11] In a study evaluating the potential impact of the reverse algorithm on syphilis diagnosis, approximately 140.000 serum samples screened initially with ELISA were analyzed. Samples were collected from patients residing in areas of low or high syphilis prevalence. About 3.4% (4,834) of samples tested by ELISA were reactive, and 56.7% (2,743) of these were nonreactive by RPR. Discordant samples were further tested with FTA-ABS, and 31.6% (833) were found to be nonreactive, suggesting false-positive ELISA results.[12] Another study comparing the screening superiority of ELISA versus RPR demonstrated several advantages of ELISA over conventional flocculation screening tests. Unlike RPR, ELISA provides more objective results and is not affected by the prozone phenomenon or the stage of syphilis infection. However, ELISA does not achieve 100% sensitivity and specificity, cross-reactivity can occur, and combined testing is recommended.[13] In a related study examining 1000 patient samples, it was emphasized that the conventional algorithm should ideally not be used for screening and that treponemal and nontreponemal tests should be applied together, with discordant results, if possible, evaluated with a second treponemal test. The study also highlighted that treponemal tests used in the reverse algorithm may produce false-positive results, especially in older patients, and that ELISA reactivities with COI <20 U were mostly negative when checked with FTA-ABS, yet treponemal tests should still be selected as the first-line test.[14] It is well known that nontreponemal test performance depends on the stage of infection. Sensitivity can decrease to 60–70% in the primary stage, potentially leading to missed asymptomatic cases in early infection.[15] In our study, the lower RPR positivity compared to treponemal tests aligns with this information, suggesting that using nontreponemal tests alone in screening may result in underdiagnosis and that treponemal-based screening is more appropriate. Another advantage of the reverse algorithm is the potential for automation. New-generation automated treponemal tests, such as Alinity, Architect, and Elecsys, have been reported to be successful in blood donor screening, offering low false-positive rates and high workflow efficiency.[16]

Limitations

However, limitations of the reverse algorithm should also be considered. Despite the high sensitivity of treponemal screening tests, false positivity has been reported in older adults, patients with autoimmune diseases, pregnancy, and certain viral infections.[7] In our study, higher ELISA positivity in patients aged 60 and older may support the notion of age-related reduced specificity. The 20 patients who were positive across all three tests (RPR, TPHA, and ELISA) were observed in all age groups, with the youngest group being 0–18 years and the oldest group being over 60 years. Additionally, a recent evaluation by Ortiz et al.[17] emphasized that the reverse algorithm can create confusion for clinicians, particularly in ELISA-positive/RPR-negative samples, highlighting the necessity of reflex confirmatory testing. In the reverse algorithm, discordant results (treponemal test positive and nontreponemal test negative) may be observed. In such cases, a reflex confirmatory test is recommended, meaning that the laboratory automatically performs a second, different treponemal assay (e.g., TPPA or FTA-ABS) on the same sample to confirm true infection and rule out false-positive screening results without requiring a new sample or separate physician request.[17] A major limitation of our study is the lack of additional clinical data accompanying laboratory results. Interpretation of treponemal test positivity was challenging without information on treatment history, previous test results, and clinical findings. Moreover, the inability to perform FTA-ABS, the gold standard confirmatory test, limited the evaluation of potential false-positive or false-negative results.

CONCLUSION

Overall, our study reinforces that treponemal tests are suitable as the first step in syphilis screening, that treponemal test positivity should always be confirmed with a different

second treponemal method, and that nontreponemal tests play an indispensable role in assessing active infection and monitoring treatment. Larger prospective studies evaluating algorithm performance across different age groups and clinical risk levels are warranted. A fundamental challenge in interpreting syphilis serology is that treponemal tests remain positive for life, meaning they are not sufficient alone to distinguish between past and active infection. The increasingly adopted reverse algorithm provides significant advantages for early detection of asymptomatic and latent cases. In our study, the notable frequency of ELISA-positive/RPR-negative samples aligns with the sensitivity advantage offered by the reverse algorithm, which is also strongly supported by recent literature.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Ondokuz Mayıs University Ethics Committee for Clinical Researches (Date: 15.10.2025, Decision No: 2025/492).

Informed Consent

As this was a retrospective study, formal written informed consent was not required and was therefore not obtained.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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Author Contributions

Concept: CÇ, ATY; Design: CÇ, YTÇ, AB; Control: DGV, KB; Resources: AB, YTÇ; Materials: ATY, CÇ, YTÇ; Data Collection and/or Processing: AB, KB, DGV; Analysis and/or Interpretation: AB, KB, YTÇ; Literature Review: CÇ, ATY; Writing the Article: CÇ, KB, ATY; Critical Review: CÇ, YTÇ, DGV, ATY, KB, AB.

REFERENCES

1. Peeling RW, Mabey D, Chen XS, Garcia PJ. Syphilis. *Lancet*. 2023; 402(10398):336-346. doi:10.1016/S0140-6736(22)02348-0
2. Forrestel AK, Kovarik CL, Katz KA. Sexually acquired syphilis: laboratory diagnosis, management and prevention. *J Am Acad Dermatol*. 2020;82(1):17-28. doi:10.1016/j.jaad.2019.02.074
3. Şamlıoğlu P. Evaluation of one-year rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) results in a tertiary public hospital. *Clin Med Sci*. 2018;6(2):9-11. doi:10.5222/TMCD.2018.0
4. Basgalupp S, Dornelles T, Pedrotti L, et al. Diagnostic properties of different serological methods for syphilis testing in Brazil. *Diagnostics (Basel)*. 2025;15(12):1448. doi:10.3390/diagnostics15121448
5. Simčič S, Potočnik M. Serological diagnosis of syphilis: a comparison of different diagnostic methods. *Acta Dermatovenerol Alp Pannonica Adriat*. 2015;24(2):17-20. doi:10.15570/actaapa.2015.6
6. Vural DG, Ateş E, Çaycı YT, Bilgin K, Birinci A. Evaluation of five-year rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) results in a university hospital. *Value Health Sci*. 2024; 14(2):198-203. doi:10.5222/ValueHealthSci.2024.198
7. Karaca Y, Çöplü N, Gözalan A, Öncül Ö, Akın L, Esen B. Establishment of an algorithm for serological testing of syphilis identification. *Mikrobiyol Bul*. 2010;44(1):35-45. doi:10.5578/mb.8891
8. Binnicker MJ, Jespersen DJ, Rollins LO. Direct comparison of the traditional and reverse syphilis screening algorithms in a population with a low prevalence of syphilis. *J Clin Microbiol*. 2012;50(1):148-150. doi:10.1128/JCM.05636-11
9. Sönmez C. Evaluation of RPR and TPHA test results and diagnostic algorithms used in syphilis diagnosis. *Turk J Med Sci*. 2018;48(1):72-77. doi:10.3906/sag-1706-100
10. Ziver T, Yüksel P, Güngördü Z, İzmirli S. Evaluation of rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) test results used in the diagnosis of syphilis infections between 2005 and 2010. *Turk J Hyg Exp Biol*. 2011;68(1):1-7. doi:10.5505/TurkHijyen.2011.88700
11. 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(6):700-708. doi:10.1086/655832
12. Radolf JD, Bolan G, Park IU, et al. Discordant results from reverse sequence syphilis screening—five laboratories, United States, 2006-2010. *MMWR Morb Mortal Wkly Rep*. 2011;60(5):133-157.
13. Negash M, Wondmagegn T, Geremew D. Comparison of RPR and ELISA with TPHA for the diagnosis of syphilis: implication for updating syphilis point-of-care tests in Ethiopia. *J Immunol Res*. 2018; 2018:2978419. doi:10.1155/2018/2978419
14. Nah EH, Cho S, Kim S, Cho HI, Chai JY. Comparison of traditional and reverse syphilis screening algorithms in medical health checkups. *Ann Lab Med*. 2017;37(6):511-515. doi:10.3343/alm.2017.37.6.511
15. Clement ME, Okeke NL, Hicks CB. Treatment of syphilis: a systematic review. *JAMA*. 2014;312(18):1905-1917. doi:10.1001/jama.2014.13259
16. Cheng A, Das A, Styles CE, Naing Z, Rawlinson WD, Gosbell IB. Improved efficiency using sequential automated immunoassays for syphilis screening in blood donors. *J Clin Microbiol*. 2024;62(8): e0047624. doi:10.1128/jcm.00476-24
17. Ortiz DA, Shukla MR, Loeffelholz MJ. The traditional or reverse algorithm for diagnosis of syphilis: pros and cons. *Clin Infect Dis*. 2020; 71(Suppl 1):S43-S51. doi:10.1093/cid/ciaa307