Clin. Lab. 2027;73:XXX-XXX

ORIGINAL ARTICLE

Comparative Evaluation of Syphilis Screening: Traditional, Reverse, and ECDC Dual-Treponemal Algorithms

Baris Can [1, 2], Reyyan Aktas [1, 2], Ozgur Yanilmaz [2], Arzu Ilki [1, 2]

[1] *Department of Medical Microbiology, School of Medicine, Marmara University, Istanbul, Türkiye*
[2] *Department of Medical Microbiology, Pendik Training and Research Hospital, Marmara University, Istanbul, Türkiye*

ABSTRACT

Background: **Serologic testing is central to the diagnosis of syphilis; however, the optimal screening algorithm remains controversial. This study compared the traditional algorithm (non-treponemal screening with treponemal confirmation), the reverse sequence algorithm (treponemal test-first), and the European Center for Disease Prevention and Control (ECDC)´s dual-treponemal strategy using a predefined patient-level classification framework.**

Methods: **Serum samples from 1,000 patients were tested in parallel using a non-treponemal assay (rapid plasma reagin, RPR), a treponemal chemiluminescent immunoassay (CMIA; ARCHITECT Syphilis TP), and the *Treponema pallidum* hemagglutination assay (TPHA). The primary endpoint was algorithm-level positivity, defined a priori as concurrent CMIA and TPHA reactivity. Secondary analyses evaluated single-assay performance metrics relative to TPHA. An exploratory analysis was also performed to describe test reactivity patterns in HIV-positive individuals.**

Results: **CMIA was reactive in 18.4% of samples (n = 184), TPHA in 14.8% (n = 148), and RPR in 5.5% (n = 55). At the patient level, the reverse sequence and ECDC dual-treponemal algorithms were fully concordant, identifying 148 true positives and 852 true negatives, with no false-positive or false-negative results under the predefined classification framework. In contrast, the traditional RPR-first algorithm identified only 55 positive cases, reflecting the limited sensitivity of non-treponemal screening. When compared with TPHA, CMIA demonstrated 100% sensitivity and 95.8% specificity (36 CMIA-positive/TPHA-negative results), whereas RPR showed 37.2% sensitivity and 100% specificity.**

Conclusions: **In a parallel-testing workflow with universal treponemal confirmation, the reverse sequence and ECDC dual-treponemal algorithms outperform the traditional algorithm and yield identical patient-level classifications within this framework. These findings reflect operational concordance rather than independent diagnostic accuracy against an external gold standard. Treponemal-first algorithms should be preferred for routine syphilis screening, while RPR remains valuable for disease staging and treatment monitoring but is suboptimal as a standalone screening test.**

(Clin. Lab. 2027;73:xx-xx. DOI: 10.7754/Clin.Lab.2026.251226)

Correspondence:
Baris Can
Department of Medical Microbiology
School of Medicine
Marmara University
Istanbul
Türkiye
Phone: +90 5327751752
Email: drbarisc@hotmail.com
ORCID: 0000-0002-1966-4240

Manuscript accepted January 30, 2026

KEYWORDS

syphilis, diagnostic algorithms, treponemal tests, screening, sensitivity, specificity

LIST OF ABBREVIATIONS

CMIA - Chemiluminescent microparticle immunoassay
TPHA - Treponema pallidum hemagglutination assay

RPR - Rapid plasma reagin
ECDC - European Center for Disease Prevention and Control
STI - Sexually transmitted infection
HIV - Human immunodeficiency virus
PPV - Positive predictive value
NPV - Negative predictive value
CI - Confidence interval
K - Cohen's kappa
TP - True positive
TN - True negative
FP - False positive
FN - False negative
S/CO - Signal-to-cutoff ratio
QC - Quality control

INTRODUCTION

Syphilis, caused by the spirochete *Treponema pallidum*, remains a significant public health challenge despite the availability of effective antibiotic therapy. Its incidence has increased in many regions worldwide, and the disease's protean clinical manifestations - ranging from primary and secondary to latent and tertiary stages - continue to complicate timely diagnosis. In addition to direct morbidity, syphilis is associated with adverse pregnancy outcomes and increased HIV transmission, highlighting the ongoing need for early, accurate, and scalable diagnostic strategies [1-4].

Because *T. pallidum* cannot be routinely cultured *in vitro*, laboratory diagnosis relies almost exclusively on serologic testing. Non-treponemal assays, such as the rapid plasma reagin (RPR) test, detect antibodies directed against lipoidal antigens released during tissue damage. These tests are inexpensive, rapid, and provide quantitative titers that typically decline following effective therapy, making them valuable for disease staging and treatment monitoring. However, their diagnostic sensitivity is limited in very early infection and late latent disease, and results may be affected by biological false-positive reactions, the prozone phenomenon, or immunosuppression.

In contrast, treponemal assays - including the *Treponema pallidum* hemagglutination assay (TPHA) and chemiluminescent microparticle immunoassays (CMIA) - detect antibodies directed against specific *T. pallidum* antigens. These tests generally offer higher analytical sensitivity and specificity for infection detection; however, treponemal antibodies often persist after successful treatment, limiting their utility for monitoring therapeutic response [5,6].

To integrate the complementary strengths of these assays, clinical laboratories employ structured diagnostic algorithms. In the traditional algorithm, screening is performed using a non-treponemal test (e.g., RPR), followed by confirmatory testing with a treponemal assay (e.g., TPHA). The reverse sequence algorithm inverts this order by using a treponemal immunoassay (e.g., CMIA) for initial screening, with a non-treponemal test applied to assess disease activity; discordant results are resolved using a second treponemal assay. The European Center for Disease Prevention and Control (ECDC)´s dual-treponemal strategy recommends screening with one treponemal assay and confirming reactive results with a different treponemal test, reserving non-treponemal assays primarily for staging and treatment monitoring [3,7,8].

Algorithm performance is influenced not only by intrinsic assay characteristics but also by laboratory workflow design. In parallel-testing workflows, a predefined patient-level classification may be applied to harmonize interpretation across diagnostic strategies. When specimens are tested in parallel using RPR, CMIA, and TPHA, this classification defines positivity as concurrent CMIA and TPHA reactivity. Within such a framework, algorithm comparisons primarily reflect operational concordance and case capture rather than independent diagnostic accuracy against an external reference standard. Under these conditions, the reverse and ECDC strategies converge to identical patient-level classifications, whereas the traditional algorithm remains constrained by the limited sensitivity of its non-treponemal screening step.

The objective of this study was to compare the diagnostic performance of the traditional, reverse, and ECDC dual-treponemal algorithms in 1,000 consecutively tested serum samples. RPR, CMIA (ARCHITECT Syphilis TP), and TPHA were performed in parallel. The primary endpoint was patient-level (algorithm-level) classification, while secondary outcomes assessed assay-level performance to characterize individual test behavior. In addition, an exploratory analysis was conducted to describe test reactivity patterns in HIV-positive individuals.

MATERIALS AND METHODS

Study design and population

This cross-sectional analytical study was conducted in the Department of Medical Microbiology, Marmara University, Pendik Training and Research Hospital (Istanbul, Türkiye). A total of 1,000 consecutive serum samples submitted for syphilis serology between April 2025 and September 2025 were included. Specimens were obtained from individuals undergoing routine sexually transmitted infection (STI) screening, antenatal testing, or clinical evaluation for suspected syphilis.

Basic demographic data and HIV serostatus were extracted from laboratory records. HIV status was recorded but not incorporated into the primary endpoint; subgroup summaries were prespecified as exploratory. Clinical information such as symptomatology, syphilis stage, prior treatment history, pregnancy status, or timing of exposure was not systematically available in laboratory records and was therefore not included in the analysis.

Serological tests

All samples were tested in parallel using three assays:

1) Rapid plasma reagin (RPR): A non-treponemal rapid plasma reagin test (Monlab, Spain) was performed according to the manufacturer's instructions. Reactive sera were titrated by serial twofold dilutions, and titers ≥ 1:1 were considered reactive.

2) Chemiluminescent microparticle immunoassay (CMIA): A treponemal chemiluminescent microparticle immunoassay (ARCHITECT Syphilis TP; Abbott Diagnostics, Germany) was used to detect *Treponema pallidum*-specific antibodies. Results were expressed as signal-to-cutoff (S/CO) indices and interpreted according to the manufacturer's criteria (reactive ≥ 1.0; non-reactive < 1.0). S/CO values were recorded due to their reported correlation with confirmatory treponemal reactivity [9].

3) *Treponema pallidum* hemagglutination assay (TPHA): The *Treponema pallidum* hemagglutination assay (Linear, Spain) was performed and interpreted visually according to standard procedures.

All assays were performed on fresh or appropriately stored sera (2 - 8°C). Internal and external quality controls (QC) were conducted in accordance with manufacturer quality-assurance recommendations and institutional laboratory policies.

As a safeguard against the prozone phenomenon, which may cause false-negative non-treponemal results in the presence of high antibody titers [4,5], samples showing treponemal reactivity with non-reactive RPR results were retested using serial twofold dilutions up to 1:128; no prozone effect was detected.

CMIA-reactive/TPHA-nonreactive samples were not subjected to additional treponemal testing (e.g., TPPA or FTA-ABS) or clinical chart review, as this study was designed as a laboratory-based parallel testing evaluation using routine diagnostic data. No longitudinal follow-up samples were available for these cases.

Diagnostic algorithms evaluated

Three diagnostic algorithms were evaluated:

1) Traditional algorithm: RPR: TPHA (non-treponemal screening followed by treponemal confirmation).

2) Reverse sequence algorithm: CMIA: RPR, with TPHA used to adjudicate discordant treponemal/non-treponemal results.

3) ECDC dual-treponemal algorithm: CMIA: TPHA, with RPR reserved exclusively for disease staging and treatment monitoring [3,8].

The diagnostic workflows are summarized schematically in Figure 1.

Interpretation and classification

The primary endpoint was an algorithm-level (patient-level) classification, defined a priori as positivity requiring concurrent CMIA and TPHA reactivity. Parallel testing with CMIA, TPHA, and RPR enabled application of this definition. Under this framework, comparisons between diagnostic algorithms reflect patient-level concordance rather than independent diagnostic accuracy against an external reference standard, and the reverse sequence and ECDC dual-treponemal algorithms yield identical classifications.

Secondary (assay-level) analyses evaluated the analytical performance of individual tests relative to TPHA (e.g., CMIA vs. TPHA; RPR vs. TPHA), independent of the algorithm-level classification.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with 95% confidence intervals (CIs) using the exact Clopper-Pearson method. Agreement between algorithms was assessed using Cohen's kappa (κ) with 95% CIs and interpreted according to Landis-Koch criteria. Primary accuracy analyses were based on the predefined patient-level classification (CMIA+ and TPHA+ = positive), whereas assay-level analyses used TPHA as the reference standard. All statistical analyses were performed using SPSS v 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Overall testing outcomes

All 1,000 serum samples were tested in parallel using RPR, CMIA, and TPHA. Overall, CMIA was reactive in 18.4% of samples (n = 184), TPHA in 14.8% (n = 148), and RPR in 5.5% (n = 55). A total of 816 samples were non-reactive in both CMIA and TPHA (Table 1).

Primary analysis: patient-level classification

Using the prespecified patient-level definition (concurrent CMIA and TPHA reactivity), the reverse sequence algorithm and the ECDC dual-treponemal algorithm yielded identical results, classifying 148 true positives (TP) and 852 true negatives (TN), with no false-positive (FP) or false-negative (FN) classifications. At the patient level, the reverse sequence algorithm showed complete concordance with the ECDC dual-treponemal algorithm, with perfect agreement (Cohen's κ = 1.00).

In contrast, the traditional RPR-first algorithm identified only 55 positive cases, reflecting under-detection attributable to the lower sensitivity of non-treponemal screening in this cohort (Table 2A).

Secondary analysis: assay-level performance (vs. TPHA)

When evaluated against TPHA, CMIA demonstrated 100% sensitivity (148/148) and 95.8% specificity (816/852), with 36 CMIA-positive/TPHA-negative results. In comparison, RPR showed 37.2% sensitivity (55/148) and 100% specificity (852/852) (Table 2B). Thirty-six samples were CMIA-reactive but TPHA-nonreactive; no additional adjudication or follow-up testing was performed for these samples. Positive and negative predictive values (PPV and NPV), along with 95% confidence

Table 1. Distribution of reactive samples by test type (RPR, CMIA, TPHA).

Test type	Total group n = 1,000		HIV-positive group n = 163	
	Reactive (n)	Reactive (%)	Reactive (n = 163)	Reactive (%)
RPR	55	5.5	36	22.1
CMIA	184	18.4	104	63.8
TPHA	148	14.8	93	57.1

Table 2A. Algorithm-level diagnostic performance using predefined patient-level classification.

Algorithm	Screening approach	Sensitivity (%)	Specificity (%)	Cohen's κ (95% CI)
Traditional	RPR → TPHA	37.2	100.0	0.50 (95% CI: 0.42 - 0.58)
Treponemal-first (reverse/ECDC)	CMIA → RPR; TPHA for confirmation/adjudication	100.0	100.0	1.00 (95% CI: 1.00 - 1.00)

Patient-level classification was predefined as concurrent CMIA and TPHA reactivity (CMIA+ and TPHA+). Reverse sequence and ECDC dual-treponemal algorithms produced identical patient-level classifications in this parallel-testing design. RPR was used for disease activity assessment and staging, and for adjudication of CMIA-RPR discordance. Cohen's kappa (κ) values are reported with 95% confidence intervals.

Table 2B. Assay-level performance versus TPHA (single-assay analytical behavior).

Assay (vs. TPHA)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	2 x 2 contingency table (TPHA reference)
CMIA	100.0 (97.5 - 100.0)	95.8 (94.2 - 97.0)	80.4 (74.0 - 85.8)	100.0 (99.5 - 100.0)	148 TP/816 TN/36 FP/0 FN
RPR	37.2 (29.4 - 45.5)	100.0 (99.6 - 100.0)	100.0 (93.5 - 100.0)	90.2 (88.2 - 92.0)	55 TP/852 TN/0 FP/93 FN

TP: true positive, TN: true negative, FP: false positive, FN: false negative.
Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with 95% confidence intervals using the exact Clopper-Pearson method. PPV and NPV reflect assay performance within the observed study prevalence (14.8% TPHA positivity). Assay-level metrics describe single-test analytical behavior and differ from algorithm-level classifications that incorporate dual-treponemal confirmation.

intervals, are presented in Table 2B and reflect assay performance within the observed study prevalence.
These assay-level metrics describe single-test analytical behavior and therefore differ from algorithm-level performance, in which the ECDC dual-treponemal algorithm operationally adjudicates isolated CMIA reactivity as non-reactive at the patient level. Similar findings have been reported in diverse clinical settings [10-13].

Algorithm concordance

At the patient level, agreement between the reverse sequence algorithm and the ECDC dual-treponemal algorithm was perfect (Cohen's κ = 1.00). Agreement between the traditional RPR-first algorithm and either treponemal-first strategy was moderate (κ ≈ 0.50), driven primarily by CMIA-positive and TPHA-positive cases missed by the traditional algorithm (Table 2A).

Exploratory HIV-positive subgroup

Among the 163 HIV-positive individuals, CMIA was reactive in 63.8% (n = 104), TPHA in 57.1% (n = 93), and RPR in 22.1% (n = 36). All RPR-reactive sera in the HIV-positive subgroup were also TPHA-reactive, indicating no false-positive RPR results in this subgroup. These findings illustrate the reduced sensitivity of non-treponemal testing in the context of immunosuppression, a phenomenon that has been well described among people living with HIV and attributed to altered

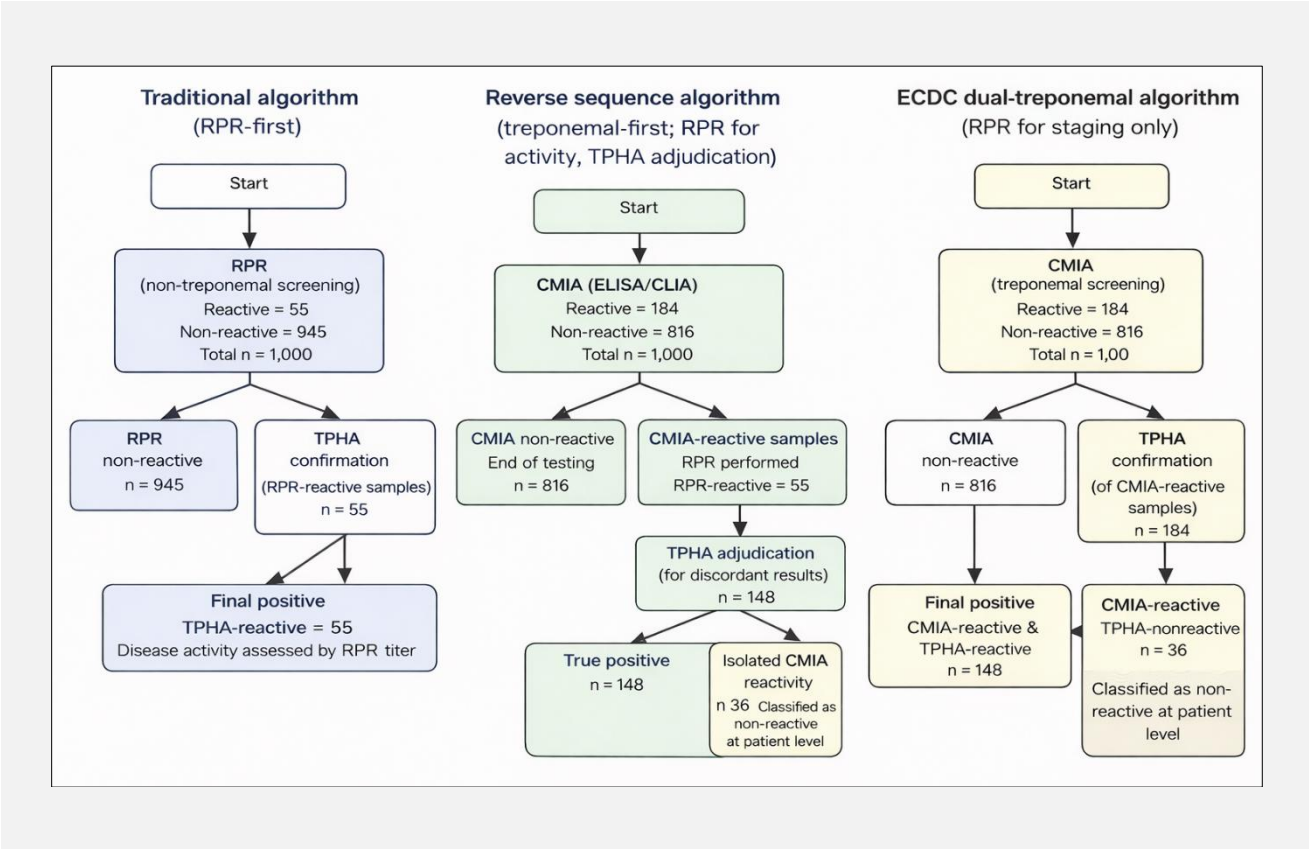

Figure 1. Diagnostic workflows for syphilis screening using the traditional (RPR-first), reverse sequence, and ECDC dual-treponemal algorithms in 1,000 serum samples.

Patient-level classification was predefined as concurrent CMIA and TPHA.

humoral immune responses [4,5,14]. These descriptive results did not alter the overall patient-level classifications (Table 1).

Quantitative RPR findings

Among RPR-reactive sera (n = 55), titers ranged from 1:1 to 1:64. The majority of samples (48/55, 87.3%) demonstrated low titers (≤ 1:8), including 21 samples with a titer of 1:1, 17 with 1:2, and 10 with 1:4 or 1:8. Higher titers (> 1:16) were observed in seven cases (12.7%), all of which were CMIA- and TPHA-reactive, consistent with active infection. Given the skewed distribution toward low titers and the limited number of high-titer results, median or geometric mean titers were not calculated. Post-treatment follow-up data were not available.

Summary of diagnostic performance

In this parallel-testing design, the reverse sequence and ECDC dual-treponemal algorithms produced identical patient-level classifications with complete concordance under the predefined framework. In contrast, the traditional RPR-first algorithm missed a substantial proportion of confirmed infections (Table 2A). The apparent reduction in CMIA specificity observed at the assay level was resolved at the algorithm level through dual-treponemal confirmation (Table 2B vs. Table 2A) [10-13, 16-19].

DISCUSSION

Accurate laboratory diagnosis of syphilis is challenged by the complex kinetics of *Treponema pallidum* antibody responses and the distinct analytical characteristics of treponemal and non-treponemal assays. In this parallel-testing design, the present study enabled a direct comparison of the traditional (RPR→TPHA), reverse sequence (CMIA→RPR with TPHA adjudication), and ECDC dual-treponemal (CMIA→TPHA; RPR for staging) algorithms using a prespecified patient-level endpoint (positive = CMIA+ and TPHA+) [3,7,8,15].

Because TPHA was systematically performed for all specimens, the reverse sequence and ECDC dual-treponemal strategies produced identical patient-level classifications, whereas the traditional RPR-first algorithm

identified only 55 positive cases. This under-detection highlights the limited sensitivity of non-treponemal screening, particularly in settings where antibody titers are low, such as early infection or late latent disease. These findings support current recommendations favoring treponemal-first approaches for initial diagnosis from an operational and workflow perspective, particularly in settings where universal treponemal confirmation is applied [3,4,8].

The low RPR reactivity observed in this cohort should be interpreted in light of the limited clinical context available. Information on symptomatology, stage of infection, prior syphilis treatment, pregnancy status, or timing of exposure was not systematically accessible. Each of these factors is known to influence non-treponemal antibody responses and may contribute to reduced RPR sensitivity, particularly in early infection, late latent disease, or previously treated individuals. In this context, the majority (approximately 87%) of RPR-reactive samples exhibited titers ≤ 1:8, a pattern commonly observed in early infection, late latent disease, or previously treated (serofast) syphilis [3-5].

At the assay level, benchmarking against TPHA demonstrated 100% sensitivity and 95.8% specificity for CMIA, with reduced specificity driven by isolated CMIA reactivity (CMIA+/TPHA-), while RPR exhibited high specificity but markedly lower sensitivity (37.2%) (Table 2B). Importantly, this apparent reduction in CMIA specificity reflects screen-only behavior. Once dual-treponemal confirmation is applied - either selectively in the reverse sequence algorithm or systematically in the ECDC dual-treponemal algorithm - isolated reactive screening results are operationally adjudicated, yielding complete patient-level concordance within the predefined classification framework.

Comparable patterns have been reported across blood donor and antenatal screening cohorts, demonstrating improved case detection and operational efficiency with treponemal-first workflows [10-13,16-19]. In particular, structured adjudication of isolated reactive chemiluminescent assays and sequential automated immunoassay strategies have been shown to enhance diagnostic clarity without increasing false-positive rates [16-18]. These findings are consistent with current international guidance from the ECDC and CDC, which increasingly emphasize reverse sequence and dual-treponemal algorithms for initial diagnosis, particularly in settings where non-treponemal assays are reserved for staging and treatment monitoring [3,4,8].

Isolated CMIA reactivity (CMIA+/TPHA-) may reflect analytical false positivity, very early infection prior to full treponemal seroconversion, previously treated syphilis, or nonspecific assay reactivity, as previously described in comparative treponemal assay evaluations [10-13,16-18]. Because no alternative treponemal testing, clinical correlation, or longitudinal follow-up was available, these cases could not be definitively adjudicated. Importantly, under both the reverse sequence and ECDC dual-treponemal strategies, such isolated CMIA reactivity is systematically resolved as non-reactive at the patient level, preventing misclassification.

The perfect agreement observed between the reverse sequence and ECDC dual-treponemal strategies (κ = 1.00), contrasted with only moderate agreement between the traditional and treponemal-first algorithms, reflects the consistently lower performance of the traditional algorithm. These findings align with previous evaluations conducted in diverse clinical populations [7, 15].

In the exploratory HIV-positive subgroup, reduced non-treponemal reactivity was observed despite preserved treponemal test positivity. This pattern is consistent with published guidelines and reviews recommending treponemal-first screening in populations with prevalent immunosuppression, where non-treponemal assays may demonstrate attenuated sensitivity [4,5,14].

From an operational perspective, laboratories may implement either the ECDC dual-treponemal strategy or a reverse sequence algorithm that reserves TPHA for discordant results. When appropriate adjudication is enforced, both approaches yield equivalent patient-level outcomes, with potential savings in reagent use and labor in high-throughput laboratory settings [17].

Several limitations should be acknowledged. Detailed clinical information, including symptomatology, stage of infection, prior syphilis treatment, pregnancy status, and timing of exposure, was not systematically available, which limits interpretation of non-treponemal test reactivity at the individual level. TPHA, although widely accepted, is not an absolute gold standard and may fail to detect very early infection. Incorporation of clinical data or molecular testing could further strengthen case adjudication. In addition, neurosyphilis requires cerebrospinal fluid-based diagnostic approaches, for which test performance characteristics differ substantially from those of serum assays [20]. Finally, post-treatment follow-up data were unavailable. Nevertheless, the continued importance of routine antenatal screening is reaffirmed by recent policy updates [21].

CONCLUSION

In a parallel-testing design with universal treponemal confirmation, the reverse sequence and ECDC dual-treponemal algorithms yielded identical patient-level classifications with complete concordance, clearly outperforming an RPR-first strategy in case identification within this predefined framework. These findings reflect operational performance and classification concordance rather than independent diagnostic accuracy against an external gold standard. For routine syphilis diagnosis, treponemal-first screening should be preferred - using the ECDC approach where systematic dual-treponemal confirmation is feasible, and the reverse sequence algorithm where operational efficiency and cost considerations are paramount. While RPR remains indispensable for disease staging and post-treat-

ment monitoring, it should not be used as a standalone screening test.

Source of Support:
This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval Statement:
Ethical approval for this study was obtained from the Marmara University School of Medicine Clinical Research´s Ethics Committee (approval number: 09.2025. 25-0197; date: March 26, 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Declaration of Generative AI in Scientific Writing:
The authors declare that no generative artificial intelligence was used in the preparation of this manuscript.

Declaration of Interest:
The authors declare no conflicts of interest.

References:

1. European Centre for Disease Prevention and Control. Syphilis-Annual epidemiological report for 2023. Stockholm: ECDC; 2025. https://www.ecdc.europa.eu/en/publications-data/syphilis-annual-epidemiological-report-2023

2. Treger RS, Menza TW, Truong TT, Lieberman JA. Advances in syphilis diagnostics to address the 21st-century epidemic. Clin Chem 2025;71(9):935-48. (PMID: 40510004)

3. Janier M, Unemo M, Dupin N, Tiplica GS, Potočnik M, Patel R. 2020 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol 2021 Mar;35(3):574-88. (PMID: 33094521)

4. Papp JR, Park IU, Fakile Y, Pereira L, Pillay A, Bolan GA. CDC laboratory recommendations for syphilis testing, United States, 2024. MMWR Recomm Rep 2024;73(1):1-32. (PMID: 38319847)

5. Luo Y, Xie Y, Xiao Y. Laboratory diagnostic tools for syphilis: current status and future prospects. Front Cell Infect Microbiol 2021;10:574806. (PMID: 33628742)

6. Zarakolu P. [Recent developments in laboratory diagnosis of syphilis.] Mikrobiyol Bul 2023;57(1):141-55. (PMID: 36636853)

7. 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):511-5. (PMID: 28840989)

8. Morshed M, Vallée M, Zhou HY, et al. Canadian Public Health Laboratory Network national syphilis in-laboratory serological testing recommendations. J Assoc Med Microbiol Infect Dis Can 2025;10(2):112-26. (PMID: 40673048)

9. Demir Çuha M, Özdemir A, Evren K, et al. Correlation of treponemal chemiluminescent microparticle immunoassay screening test signal strength with reactivity of confirmatory testing. Sex Transm Dis 2022;49(6):453-7. (PMID: 35312664)

10. Kakkar B, Philip J, Mallhi RS. Comparative evaluation of rapid plasma reagin and ELISA with *Treponema pallidum* hemagglutination assay for the detection of syphilis in blood donors: a single-center experience. Hematol Transfus Cell Ther 2024;46 (Suppl):S43-S47. (PMID: 38429196)

11. 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. (PMID: 30069486)

12. Solaimalai D, Rathore S, Beck MM, et al. Enzyme-linked immunosorbent assay versus Venereal Disease Research Laboratory test and rapid plasma reagin test for screening of syphilis in pregnant women. Int J Gynaecol Obstet 2020;150(1):103-7. (PMID: 32246772)

13. Lyngdoh CJ, Ramudamu M, Agarwal M, Verma S, Prasad A. Evaluation of serological tests for the diagnosis of syphilis. Cureus 2024;16(5):e61007. (PMID: 38915997)

14. Yağcı Çağlayık D, Sili U, Tükenmez Tigen E, et al. Investigation of syphilis seropositivity in adult individuals living with HIV followed at Marmara University Pendik Training and Research Hospital, 2024. Turk Hij Den Biyol Derg 2024;81(4):431-8. https://www.turkhijyen.org/eng/jvi.aspx?pdir=turkhijyen&plng=eng&un=THDBD-04207&look4=#

15. Evren K, Berkem R, Yücel M. Evaluation of the diagnostic algorithms for serodiagnosis of syphilis. Jpn J Infect Dis 2022;75(1): 70-5. (PMID: 34193666)

16. Luo X, Xiao H, Lu Y, et al. Modified TPPA combined with western blotting facilitates syphilis diagnosis in isolated reactive treponemal chemiluminescence immunoassay sera: a prospective cohort study. BMC Infect Dis 2025;25(1):6. (PMID: 39748275)

17. 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. (PMID: 39007562)

18. Fang W, Liu L, Shi M, et al. Evaluation of MAGLUMI syphilis test for accurate detection of syphilis antibodies in blood donors and suspected syphilis cases. Front Cell Infect Microbiol 2025; 15:1578060. (PMID: 40444150)

19. Silva ÂAO, Lima AA, Vasconcelos LCM, et al. Performance assessment of treponemal and nontreponemal tests for the diagnosis of acquired syphilis. Am J Trop Med Hyg 2024;110(6):1237-44. (PMID: 38593786)

20. Xie JW, Wang M, Zheng YW, Lin Y, He Y, Lin LR. Performance of nontreponemal and treponemal tests on cerebrospinal fluid for the diagnosis of neurosyphilis: a meta-analysis. Front Public Health 2023;11:1105847. (PMID: 36817920)

21. US Preventive Services Task Force; Silverstein M, Wong JB, Davis EM, et al. Screening for syphilis infection during pregnancy: US Preventive Services Task Force reaffirmation recommendation statement. JAMA 2025;333(22):2006-12. (PMID: 40358930)