SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Microbiology

Syphilis Serology in Focus: TPHA or CMIA? A Comparative Study in a Moroccan Tertiary Care Center

A. Bouchehboun[1*], J. Farhat[1], I. Mhirig[1], T. Benhoumich[1], A. Lamrani[1], N. Soraa[1]

[1]Microbiology Laboratory, Mohammed VI University Hospital of Marrakesh

DOI: https://doi.org/10.36347/sasjm.2025.v11i05.028 | **Received:** 11.04.2025 | **Accepted:** 16.05.2025 | **Published:** 21.05.2025

***Corresponding author:** Ayoub Bouchehboun
Microbiology Laboratory, Mohammed VI University Hospital of Marrakesh

Abstract **Original Research Article**

Background: Syphilis remains a global public health concern, particularly in resource-limited settings. Serological testing is central to diagnosis, but the performance of manual versus automated assays remains under evaluation. ***Objective***: To compare the diagnostic performance and concordance between a manual Treponema pallidum hemagglutination assay (TPHA) and an automated chemiluminescent microparticle immunoassay (CMIA) in detecting anti-treponemal antibodies. ***Methods***: A retrospective study was conducted at CHU Mohammed VI of Marrakech. A total of 156 serum samples initially tested using the CMIA-based Architect i2000SR system were re-analyzed using the TPHA method. Concordance, sensitivity, specificity, and co-infections were evaluated. ***Results***: All CMIA-negative samples (n=70) were also negative on TPHA (100% concordance). Among CMIA-positive samples (n=86), 81.25% were confirmed positive by TPHA, 8.75% equivocal, and 10% negative. VDRL was negative in 87.21% of CMIA-positive samples. Co-infections were present in 55.81%, most commonly CMV (30.56%) and hepatitis B (29.17%). CMIA showed a sensitivity of 88.6% and specificity of 100% versus TPHA. ***Conclusion***: CMIA offers superior sensitivity and operational efficiency, while TPHA remains valuable where automation is limited. A combined testing approach may optimize diagnostic accuracy.

Keywords: Syphilis, Serology, TPHA, CMIA, Morocco, Diagnostic Concordance.

INTRODUCTION

Laboratory diagnostics play a vital role in the detection, monitoring, and management of infectious diseases. Ensuring high quality in laboratory processes requires rigorous standards in specimen handling, analytical accuracy, and result validation. These standards underpin the reliability of test results and the effectiveness of clinical decision-making [1, 2].

Serological testing, a cornerstone of infectious disease diagnosis, detects antibodies such as IgG or IgM in serum samples. Recent technological advancements have transitioned serological platforms from manual to fully automated systems, improving analytical precision and workflow capacity [3, 4].

Syphilis, caused by Treponema pallidum, remains a diagnostic challenge due to its diverse manifestations and potential for asymptomatic carriage. Serological testing is essential, especially where direct detection methods are unavailable [5]. A wide array of tests, including manual hemagglutination assays like TPHA and automated immunoassays like CMIA, are used in clinical settings [6, 7].

Given the differences in methodology, automation, and analytical sensitivity, it is essential to compare these assays to ensure optimal diagnostic accuracy. This study aims to evaluate the concordance between TPHA and CMIA in the serological diagnosis of syphilis within a tertiary care setting.

MATERIALS AND METHODS

Study Design and Setting

A retrospective comparative study was conducted at the Microbiology Laboratory, Arrazi Hospital, CHU Mohammed VI, Marrakech, Morocco, between March 28 and May 21, 2022.

Sample Selection

Inclusion Criteria

Patients hospitalized at Arrazi Hospital – CHU Mohammed VI, or outpatients, who had already undergone syphilis testing using the chemiluminescence

method on the ARCHITECT i2000SR analyzer from venous blood (serum or plasma).

Exclusion Criteria

All biological fluids other than serum or plasma (e.g., cerebrospinal fluid, synovial fluid, etc.) were excluded from syphilis testing using the chemiluminescence method on the ARCHITECT i2000SR analyzer, due to the non-validity of this method for such specimens. Samples with hemolysis or insufficient volume were excluded.

Assays Used:

- CMIA (Architect i2000SR): Automated two-step chemiluminescent assay detecting IgG and IgM against *T. pallidum*.
- TPHA (Bio-Rad 72503): Manual hemagglutination assay using sensitized avian erythrocytes to detect anti-treponemal antibodies.

Data Analysis

Statistical analyses were performed using SPSS v25. Concordance, sensitivity, and specificity were calculated.

Ethical Approval: Ethical approval was granted by the institutional authorities. Data were anonymized.

RESULTS

- **Study Population**

A total of 156 serum samples were included. The sex distribution showed a predominance of males (60.54%), yielding a sex ratio of 1.53. The most represented age groups were 60-80 years (35%) and 40-60 years (30,85%). Table 1 summarizes the age distribution of the study population, indicating a predominance of individuals aged 40–80 years.

Table 1: Age Distribution of Study Population.

Age Group	Percentage (%)
<1 year	1.9
20–40 years	24.51
40–60 years	30.85
60–80 years	35.0
>80 years	1.29

- **CMIA and TPHA Concordance**

All 70 samples that tested negative by CMIA were also negative by TPHA, indicating a perfect concordance for negative results. Among the 86 CMIA-positive samples, 70 were TPHA-positive (81.25%), 7 were equivocal (8.75%), and 8 were TPHA-negative (10.00%). We notice complete agreement among negative samples and 81.25% concordance among positive results as showed on table 2.

Table 2: The concordance between CMIA and TPHA results

CMIA Result	TPHA Result	n	Percentage (%)
Positive	Positive	70	81.25
Positive	Equivocal	7	8.75
Positive	Negative	8	10.0
Negative	Negative	70	100.0

- **Overall Concordance** = 139/156 = 89.1%
- **Kappa Coefficient (κ)** = 0.78, 95% CI [0.68–0.88], $p < 0.001$

Diagnostic Performance (CMIA vs TPHA)

Taking TPHA as the reference:

Table 3: Diagnostic Performance of CMIA (vs TPHA)

Metric	Value (95% CI)
Sensitivity	88.6% (79.5–94.1%)
Specificity	100% (94.8–100%)
PPV	100%
NPV	88.6%

Signal/Cut-Off(S/CO) Analysis

- Mean S/CO (TPHA-positive samples): 18.5
- Mean S/CO (TPHA-equivocal/negative): 9.2
- **Difference Statistically Significant** (Shapiro-Wilk $p > 0.05$, t-test $p < 0.001$)

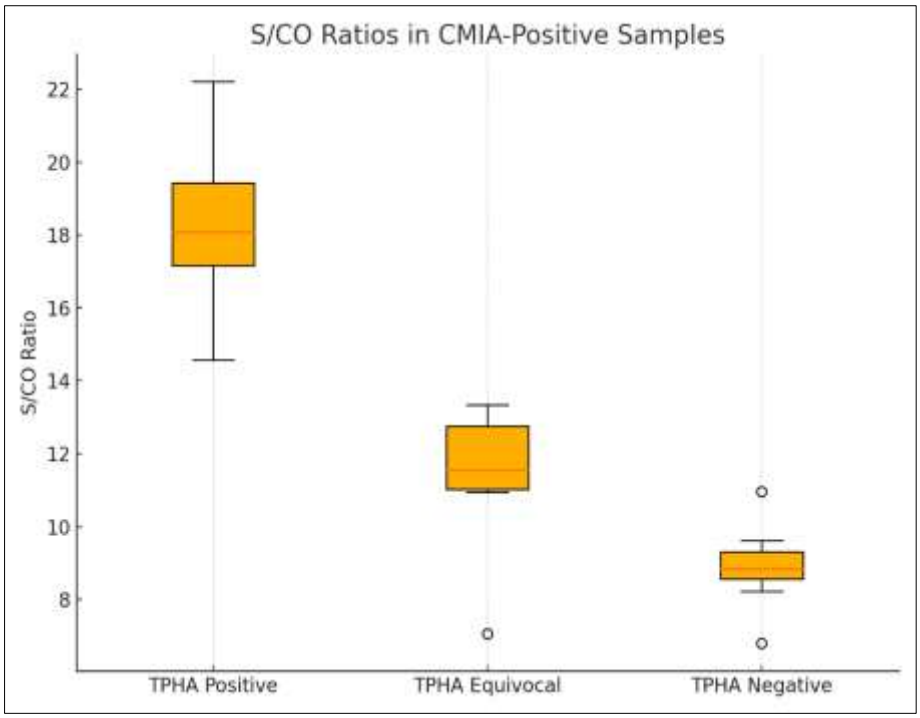

Figure 1: Boxplot - S/CO Ratios in CMIA-Positive Samples

This figure represents the distribution of Signal-to-Cutoff (S/CO) ratios in three groups: TPHA-positive, TPHA-negative, and TPHA-equivocal CMIA-positive samples. Lower S/CO values were significantly associated with discordant results ($p < 0.001$).

- **VDRL Test Results**

Among CMIA-positive patients, the non-treponemal VDRL test was positive in 12.79% (11/86) and negative in 87.21% (75/86). Results as showed in table 4.

Table 4: Table showing VDRL test results among CMIA-positive cases

VDRL Result	n	Percentage (%)
Positive	11	12.79
Negative	75	87.21

- **Co-Infections in Syphilis-Positive Patients**

Co-infection was observed in 55.81% of CMIA-positive individuals. The most frequently identified pathogens were CMV (30.56%), hepatitis B virus (29.17%), and Epstein-Barr virus (16.67%). Other common infections included rubella and toxoplasmosis. Different co-infection are listed in table 5.

Table 5: The frequency of co-infections detected in CMIA-positive individuals, with CMV and hepatitis B being the most frequent

Infection	Percentage among co-infected (%)
CMV	30.56
Hepatitis B	29.17
EBV	16.67
Rubella	12.5
Toxoplasmosis	11.11

DISCUSSION

This study aimed to evaluate the concordance between two treponemal serological assays—TPHA and CMIA—for the diagnosis of syphilis. Our findings demonstrate a high degree of agreement between the two methods, particularly in samples that tested negative by CMIA, where 100% were also non-reactive by TPHA, indicating strong specificity and consistent rule-out capacity between the two techniques [8, 9].

Among the CMIA-positive samples, 81.25% were confirmed by TPHA, while the remaining showed either equivocal (8.75%) or non-reactive (10%) TPHA results. This discrepancy highlights a known limitation of manual hemagglutination assays such as TPHA, which may have reduced sensitivity, particularly in early or low-titer infections. Previous studies have similarly reported lower sensitivity for TPHA compared to CMIA,

especially in early syphilis or in patients with serologic scars [10, 11].

A reverse screening algorithm—using CMIA as an initial test followed by confirmation with TPHA or non-treponemal testing—is recommended for optimal diagnostic accuracy, particularly in low-prevalence areas [12].

CMIA, an automated chemiluminescent microparticle immunoassay, demonstrated superior analytical sensitivity and operational advantages. Its automation, reduced turnaround time, and enhanced reproducibility make it particularly well suited for high-volume laboratories [13]. Additionally, CMIA has been shown to detect both IgM and IgG antibodies, which improves its capacity to identify both recent and past infections [14]. This broader detection capability may explain the higher number of CMIA-positive but TPHA-negative results in our study.

Nevertheless, TPHA remains a valuable tool, particularly in resource-limited settings where automated platforms may not be available. Its ease of implementation and cost-effectiveness make it a viable alternative when economic or logistical constraints exist. Moreover, the manual visual readout of TPHA, while subjective, can still provide reliable results when performed by trained personnel [15].

These findings support the integration of both methods within a diagnostic algorithm. In line with current recommendations, a reverse screening algorithm—starting with a treponemal assay (e.g., CMIA) followed by confirmatory testing (e.g., TPHA or another treponemal/non-treponemal assay)—may offer an optimal balance between sensitivity and specificity, especially in low-prevalence settings [16].

Limitations

This study had several limitations. First, its retrospective design and single-center setting may limit the generalizability of the findings. Second, the absence of clinical data or confirmatory non-treponemal testing (e.g., RPR/VDRL) prevented us from correlating serological profiles with disease stage or activity. Third, the study relied on stored serum samples, and antibody titers may degrade slightly over time despite proper storage.

CONCLUSION

In conclusion, both CMIA and TPHA demonstrated high diagnostic performance in detecting syphilis, with CMIA showing greater sensitivity and operational efficiency. TPHA remains a reliable manual alternative, particularly in settings where automated systems are unavailable. These findings highlight the complementary role of both assays within a multi-tiered diagnostic strategy for syphilis.

REFERENCES

1. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med. 2006;44(6):750–759.
2. Quality indicators in laboratory medicine: from theory to practice. Biochem Med (Zagreb). 2017;27(3):321–329.
3. Syphilis. Nat Rev Dis Primers. 2017;3:17073.
4. Evaluation of automated immunoassays for syphilis diagnosis: a comparison of analytical performances. Braz J Infect Dis. 2020;24(2):91–96.
5. Laboratory diagnosis and interpretation of tests for syphilis. Clin Microbiol Rev. 1995;8(1):1–21.
6. Which algorithm should be used to screen for syphilis? Curr Opin Infect Dis. 2012;25(1):79–85.
7. Novel Treponema pallidum Serologic Tests: A Paradigm Shift in Syphilis Screening for the 21st Century. Clin Infect Dis. 2010;51(6):700–708.
8. 2020 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol. 2021;35(1):49–55.
9. Syphilis testing algorithms using treponemal tests for initial screening—Four laboratories, New York City, 2005–2006. MMWR Morb Mortal Wkly Rep. 2008;57(32):872–875.
10. Evaluation of the Determine Syphilis TP assay as a point-of-care test. J Clin Microbiol. 2010;48(12):4652–4655.
11. Acquired syphilis in adults. N Engl J Med. 1992;326(16):1060–1069.
12. Performance of treponemal tests for the diagnosis of syphilis. Clin Infect Dis. 2019;68(6):913–918.
13. Guidelines for serological testing for syphilis. Sex Transm Infect. 2000;76(5):403–405.
14. Incidence and risk factors for serological treatment failure in HIV-negative persons with early syphilis in China. Clin Infect Dis. 2014;58(5):659–665.
15. Discordant results from reverse sequence syphilis screening—Five laboratories, United States, 2006–2010. MMWR Morb Mortal Wkly Rep. 2011;60(5):133–137.
16. Centers for Disease Control and Prevention. Discordant results from reverse sequence syphilis screening—Five laboratories, United States, 2006–2010. MMWR Morb Mortal Wkly Rep. 2011;60(5):133–137