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Screening for Syphilis Incidence in a Tertiary Care Facility: A Reverse Algorithm Cross-Sectional Investigation

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ABSTRACT

Background and Aim: Syphilis continues to present a major public health challenge globally, with serologic testing central to detection and control efforts. Comparative evaluation of screening algorithms, particularly the performance of automated Chemiluminescent Microparticle Immunoassay (CMIA) versus Rapid plasma reagin (RPR) using *Treponema (T.) pallidum* hemagglutination assay (TPHA) as the reference standard, is critical for optimizing diagnostic accuracy. This study aimed to investigate syphilis prevalence and assesse the efficacy of reverse screening strategies in a high-volume academic tertiary care center.

Materials and Methods: The study was conducted from July 2024 to March 2025 at SRM Medical College Hospital and Research Centre. Individuals presenting to the outpatient department were screened using Abbott ARCHITECT Syphilis TP (CMIA). Positive samples underwent RPR testing, followed by TPHA confirmation.

Results & Conclusion: Out of 4312 samples, 52 (1.2%) were CMIA positive. Among these, 75% were male and 25% were female, with one case of congenital syphilis. Of the CMIA-positive cases, 35 (67.3%) were RPR positive and 34 (32.7%) were TPHA confirmed. Among the 17 RPR-negative samples, 4 (23.52%) were TPHA positive and 13 (76.48%) were negative. The study found low syphilis prevalence (1.2%) among the screened population. A significant proportion of RPR-negative but TPHA-positive cases underscore the need for combined testing strategies. These findings highlight the importance of comprehensive serologic screening, continued surveillance, and targeted public health interventions.

Keywords: Chemiluminescent Microparticle Immunoassay, Hemagglutination assay, Rapid Plasma Reagin, Syphilis, *Treponema pallidum*

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1. Introduction

Sexually transmitted infections (STIs) remain a major global public health concern. The World Health Organization (WHO) periodically estimates the global burden of four curable STIs—syphilis, trichomoniasis, gonorrhea, and chlamydia—and aims to eliminate them as public health threats by 2030 through expanded access to evidence-based prevention and treatment services **(1)**. Syphilis, caused by *Treponema (T.) pallidum* subsp. *pallidum*, is transmitted sexually and vertically during pregnancy. Known as the “great imitator,” it presents with diverse clinical symptoms that resemble other diseases and can involve multiple organ systems, including the central nervous, ocular, and otic systems

(2). Congenital syphilis contributes significantly to stillbirths, miscarriages, and neonatal morbidity, particularly among high-risk populations such as sex workers (3). Globally, around six million new cases occur annually, with prevalence rates of 1.7 per 1,000 women and 1.6 per 1,000 men aged 15–49 years (4).

Serologic testing remains the cornerstone of syphilis diagnosis. Non-treponemal tests (NTTs) such as the Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) tests are commonly used for the screening but show limited sensitivity in early and late disease. Treponemal tests (TTs), including *T. pallidum* hemagglutination assay (TPHA) and particle agglutination assay (TPPA), offer higher sensitivity but cannot distinguish active from past infections. NTT sensitivity ranges from 62–78% in early syphilis to nearly 100% in secondary stages, while TT sensitivity typically exceeds 95% (5).

Two diagnostic algorithms are widely used: the Traditional Algorithm (TA) and the Reverse Algorithm (RA). TA begins with an NTT and confirms reactive results with a TT, while RA starts with a TT—often an automated Chemiluminescent Microparticle Immunoassay (CMIA) detecting *T. pallidum* IgM and IgG antibodies—followed by an NTT for confirmation. RA improves early detection, especially in high-prevalence settings, though both yield similar rates of active infection (6).

Accurate syphilis diagnosis is critical for preventing complications such as pregnancy loss and reducing the risk of co-infections like HIV. The choice of screening approach depends on factors like prevalence, patient demographics, laboratory resources, and cost-effectiveness. TA is suitable for low-prevalence settings and small-volume labs, while RA, often automated, is preferred in high-prevalence areas for its efficiency and sensitivity (7).

The intent of the study was to assess the frequency of syphilis and compare the performance between Syphilis TP (CMIA) and RPR testing, with TPHA as the gold standard. The study provides region specific data on the performance of the reverse screening algorithm for syphilis from a tertiary care center in India. Such evidence is important because variations in regional disease prevalence and healthcare infrastructure may influence the diagnostic performance, feasibility, and patterns of discordant serology associated with reverse algorithm testing in similar clinical environments.

2. Materials and Methods

2.1 Study Design and Sample Size

This prospective study was carried out on 4,321 serum samples received at SRM Medical College and Research Centre between July 2024 and March 2025. The study included serum samples from individuals screened for syphilis.

2.2 Study Protocol

Initial serological screening was performed using the Syphilis TP CMIA. All CMIA-reactive samples underwent further testing with the RPR assay. TPHA was used as the reference standard or confirmatory test for discordant (CMIA+/RPR-) results. The CMIA (Syphilis TP) and RPR (non-treponemal test) were performed as part of routine laboratory request and the TPHA (treponemal confirmatory test) was performed on the discordant results. Chemiluminescent microparticle immunoassays (CMIAs) are modified sandwich EIAs that utilize light emission to detect target antibodies (IgM and IgG). The Abbot ARCHITECT Syphilis TP assay (Architect; Abbott Laboratories, Abbott Park, IL), which uses recombinant *T. pallidum* antigens; TpN15, TpN17, and TpN47, is optimized for the detection of early syphilis (8). Test results were interpreted according to the manufacturer's guidelines: a signal-to-cutoff (S/CO) ratio ≥1.00 was considered reactive, while an S/CO ratio <1.00 was considered non-reactive (8).

The Rapid Plasma Reagin (RPR) test was performed using the slide flocculation method with the CARBOGEN RPR Card Test kit, following the manufacturer's protocol (Tulip Diagnostics Pvt. Ltd. Goa, India) (9). This non-treponemal assay detects antilipoidal antibodies indicative of active infection. A reactive result was characterized by visible flocculation, while the absence of flocculation was considered non-reactive. RPR titers were determined by serial dilution, with the highest dilution showing a reactive result recorded as the antibody titer. A titer of 1:8 and above considered as reactive (9).

The *T. pallidum* Hemagglutination Assay (TPHA) was conducted using the Monlab TPHA Test kit (Monlab SL Selva de Mar 48 08019 BCN) based on the microtiter hemagglutination method. This treponemal assay detects specific antibodies to *T. pallidum* for confirmatory purposes. A reactive result was indicated by a diffuse mat of agglutinated cells covering the well bottom, while a non-reactive result presented as a compact cell button, sometimes with a central clearing. A titer of 1:80 and above considered as reactive (10).

3. Results and Disussion

The demographic characteristics of syphilis reactive samples including age, gender, location, risk factors, marital status, and education level are shown in Table 1.

The correlations between *T. pallidum* Hemagglutination Assay (TPHA) & Rapid Plasma Reagin (RPR) are shown in Table 2.

A cohort of 4,312 patient samples was initially analyzed using ARCHITECT Syphilis TP. Of these, 52 patients (1.2%) exhibited positive results, while 4,260 patients tested negative. The number of positive samples using Architect Syphilis TP (CMIA) over a period of 9 months has been illustrated in Figure 1. The 52 patients with positive CMIA results underwent further testing with the RPR assay, which identified 35 patients (67.3%) as reactive and 17 patients (32.7%) as negative. Subsequently, the 17 patients who tested negative on the RPR test were subjected to the TPHA assay, which yielded positive results for 4 patients (23.52%) and negative results for 13 patients (76.48%) was shown on Figure 2.

As shown in Figure1 the monthly trend analysis showed that the number of samples tested using CMIA ranged from 430 to 518 between July 2024 and March 2025. CMIA reactive cases remained relatively low, varying between 4 and 10 cases per month, with the highest reactivity observed in January 2025 (10 cases). Overall, the data indicate a stable testing volume with consistently low but detectable syphilis positivity during the study period.

Table 1. Demographic characteristics of syphilis reactive samples.

Characteristics		Number (%)
Age	18-30	25 (48%)
	31-40	17 (32.6%)
	41-50	1 (1.9%)
	51-60	7 (13.4%)
	>61	2 (3.8%)
Gender	Male	39 (75%)
	Female	13 (25%)
	Others	0
Location	OPD	46 (88.4%)
	ICU	4 (7.6%)
	IP (GYN)	2 (3.8%)
Risk factors	HIV	2 (3.8%)
	Pregnancy	1 (1.9%)
	Coronary artery diseases	3 (5.6%)
	Anonymous sexual partner	17 (32.69%)
	Substance use	10 (20%)
	Unknown cases	19 (36.53%)
Marital status	MARRIED	33 (63.4%)
	UNMARRIED	19 (36.5%)
Education	PRIMARY EDUCATION &BELOW	8 (15.3%)
	SECONDARY EDUCATION	2 (3.8%)
	COLLEGE &ABOVE	42 (80.7%)

Table 2. Correlation between *T. pallidum* Hemagglutination Assay (TPHA) & Rapid Plasma Reagin (RPR).

Test	RPR reactive	RPR non-reactive
TPHA reactive	34(65.3%)	4(7.6%)
TPHA non-reactive	1(1.9%)	13(25%)

"N=52" CMIA Reactive samples.

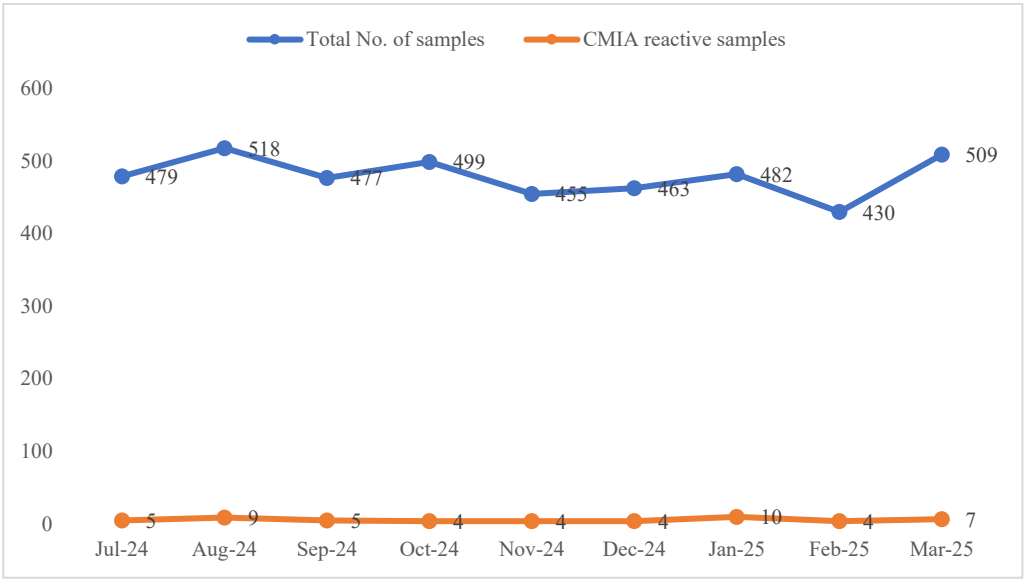

Figure 1. Trends in Syphilis detection. The graph shows trends in syphilis detection using CMIA from July 2024 till March2025. CMIA reactive samples remained relatively low, varying between 4 and 10 cases per month **(Prepared by Authors, 2026)**.

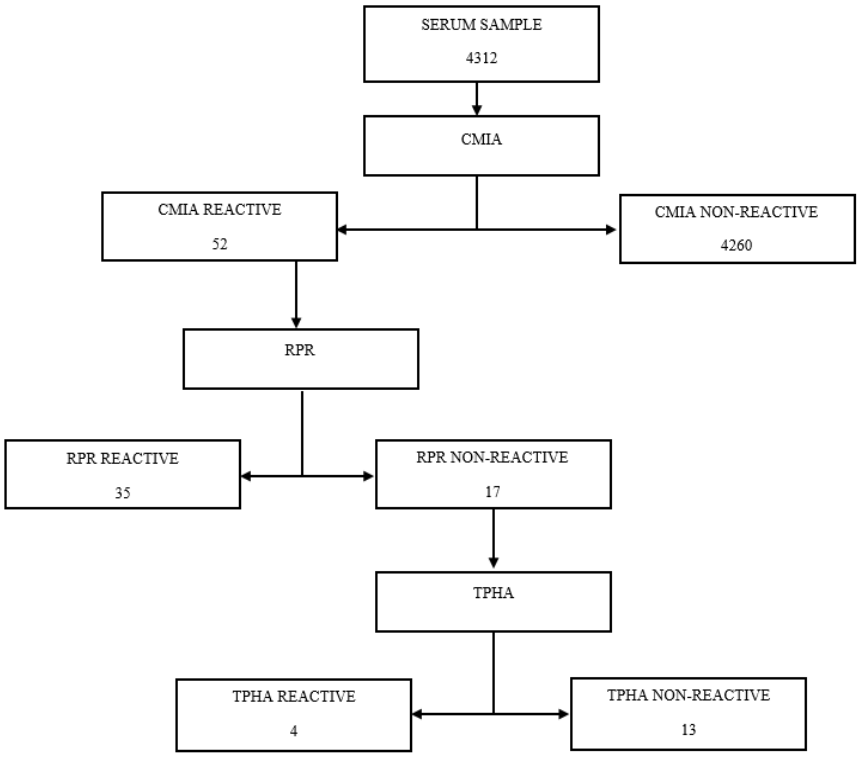

Figure 2. Study flowchart. The flowchart shows the study design **(Prepared by Authors, 2026)**.

Out of 4,312 samples submitted for CMIA testing, 52 (1.2%) were found to be reactive. The majority of individuals were under 30 years old, comprising 48% of the positive cases. This finding is consistent with studies such as Niedźwiedzka-Stadnik et al **(11)**, which reported an average age of 30-34 years for infected patients. In our study, 75% of the positive cases were male, while 25% were female. This male predominance aligns with the findings of Solaimalai et al **(12)**, who observed 82.3% male preponderance over a decade of study.

Genital ulcers were the predominant symptom, as 88.4% of the patients sought medical care either due to symptoms or asymptomatic screening. This observation was in agreement with Sardinha et al **(13)**, where 19 out of 25 patients had genital ulcers.

During our study, four patients (23.52%) exhibited a CMIA (+) RPR (−) TPHA (+) result, indicating previous syphilis infections rather than active or early-stage syphilis. This finding highlights the importance of the reverse algorithm (RA) in detecting both active and past syphilis infections. Yapar et al **(14)** demonstrated that RA is effective in identifying both active and latent syphilis cases, whereas TA might overlook such cases.

Yapar et al **(14)** also assessed the concordance between CMIA and TPHA tests, reporting high agreement between the two. Their study found 37 patients positive for both tests, while 162 were negative on both. One patient exhibited CMIA (+) and TPHA (-), which further supports the reliability of these diagnostic methods **(14)**. In our study, 13 patients showed a CMIA (+) RPR (-) TPHA (-) result, with 9 of them having a CMIA value of less than 5. Payaslıoğlu et al **(15)** suggested that latent syphilis undetected by RPR screening may still present a higher likelihood of syphilis, even with negative TPHA results.

Our study observed a 67-year-old male patient with a positive CMIA and RPR titer but a negative TPHA result. This discrepancy could be due to ongoing seroconversion or a false-negative TPHA test. Zondag et al **(16)** observed in their studies that during the early stages of syphilis, particularly in the incubation period, treponemal antibodies may not yet be detectable, resulting in negative TPHA test results. Seroconversion, the process by which these antibodies become detectable in the blood, can take up to 90 days post-infection **(16)**.

However, the TPHA test has limitations, including false positives and false negatives. Verma et al **(17)** reported a 3 false-positive rate. Additionally, Gupta et al **(18)** indicated that CMIA shows an uptrend in false positive rate under low prevalence setting. Our study observed similar findings among the 13 discordant results, supporting Gupta et al **(18)** study.

Using TPHA as the reference test, CMIA demonstrated a sensitivity of 100%, specificity of 99.7%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 100%. On the other hand, RPR showed a sensitivity of 89.7%, specificity of 100%, and NPV of 76.5%. The study performed by Terzi et al **(19)** found CMIA showed a sensitivity of 93.3% (28/30) and specificity of 99.1% (560/565), with PPV of 84.8% (28/33) and NPV of 99.6% (560/562). In comparison, RPR demonstrated 100% sensitivity (30/30) and 98.8% specificity (558/565), with PPV of 81.1% (30/37) and NPV of 100% (558/558) **(19)**.

Despite the lack of clinical history and follow-up data for patients with discordant results, discrepancies between diagnostic tests underscore the importance of considering the sensitivity and interpretation of each test. The majority of CMIA-reactive samples were also reactive in the RPR test (35 out of 52), suggesting that the need for a third treponemal test is minimal when using a highly sensitive test like CMIA. Park et al **(20)** found that the ARCHITECT Syphilis TP test showed a sensitivity of 97.3%.

RA, though more expensive, is a better algorithm suited for automation, making it ideal for high-volume testing **(21)**. Early concerns regarding its specificity have been addressed in recent studies, which show that the RA can achieve high specificity and sensitivity, surpassing TA **(17)**. Although RA is effective for detecting primary syphilis, the lifelong persistence of treponemal antibodies complicates the identification of reinfection. Therefore, accurate diagnosis of reinfection requires a comprehensive assessment incorporating non-treponemal tests, which also remain the preferred tools for monitoring treatment response **(22)**.

This study has several limitations, including substantial patient attrition, incomplete medical records, and notable deficiencies in diagnostic follow-up. The analysis was limited to blood samples and excluded patients with sexually transmitted diseases (STDs). Additionally, the study was conducted at a single center, which may further constrain the generalizability and applicability of the findings.

4. Conclusion

The applicability of the reverse algorithm, as evaluated in our study, is influenced by healthcare infrastructure and economic factors. Within our cohort, the reverse sequence algorithm demonstrated substantial effectiveness, particularly due to its ability to accommodate high testing volumes and enhance the identification of latent infections that might otherwise remain undiagnosed. Nevertheless, this diagnostic approach may result in an increased rate of false

positive outcomes, necessitating additional confirmatory testing. Despite this challenge, the algorithm offers greater diagnostic sensitivity and improves case detection. Our findings underscore the importance of balancing enhanced detection with the consequences of supplementary confirmatory tests when deploying diagnostic algorithms for optimal disease control.

5. Declarations

5.1 Acknowledgment

Not Applicable.

5.2 Ethical Considerations

All procedures were conducted in accordance with relevant laws and institutional guidelines, following approval from the appropriate institutional committees. Specifically, the research received ethical approval from both the Ethics Committee of SRM Institute of Science and Technology and the Ethics Committee of SRM Medical College Hospital and Research Centre (Approval No. SRMIEC-ST0624-1310). Informed consent was obtained from all individual participants involved in the study, ensuring their privacy rights were fully observed.

5.3 Authors' Contributions

AP: Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft; AG: Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision; MD: Writing -Review & Editing, Visualization; JSS: Writing -Review & Editing, Visualization; ASN: Writing -Review & Editing; LKV: Writing -Review & Editing, Project Administration. All authors have read and approved the final manuscript.

5.4 Conflict of Interests

The authors declare no conflict of interest.

5.5 Financial Support and Sponsorship

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

5.6 Using Artificial Intelligence Tools (AI Tools)

The authors did not use AI tools. All content, interpretations, and conclusions are solely the work of the authors.

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