GMR
The Original

COMPARATIVE EVALUATION OF RAPID PLASMA REAGIN, ENZYME-LINKED IMMUNOSORBENT ASSAY, AND CHEMILUMINESCENCE IMMUNOASSAY FOR THE DIAGNOSIS OF SYPHILIS AMONG VOLUNTARY BLOOD DONORS

Dr Aslima Banu A[1], Dr P. Subhashini[2], Dr M. Aswin Manikandan[3]

[1]Post Graduate, Department of Pathology, Sree Balaji Medical college and Hospital, email id-aslithahir996@gmail.com
[2]Professor, Department of Pathology, Sree Balaji Medical college and Hospital, email id - drsubha_path@rediffmail.com
[3]Professor, Department of Pathology, Sree Balaji Medical college and Hospital, email id- aswinm1987@outlook.com

ABSTRACT

Introduction: Syphilis remains a significant transfusion-transmissible infection requiring accurate screening among blood donors[1],[2].

Aim: To compare the diagnostic performance of Rapid Plasma Reagin (RPR) and Chemiluminescence Immunoassay (CLIA) with Enzyme-Linked Immunosorbent Assay (ELISA).

Materials and Methods: A cross-sectional study was conducted among 185 voluntary blood donors at a tertiary care center in Chennai from August 2024 to February 2026. Samples were tested using RPR, ELISA, and CLIA. Statistical analysis included Chi-square test and kappa statistics.

Results: Seropositivity was highest with CLIA (16.8%), followed by ELISA (9.7%) and RPR (5.9%). A strong association was observed between ELISA and RPR ($p < 0.001$; kappa = 0.590). CLIA showed a significant association with ELISA ($p < 0.001$) but only fair agreement (kappa = 0.325).

Conclusion: CLIA demonstrated superior sensitivity and higher detection rates compared to ELISA and RPR, making it a valuable screening tool for blood donors.

KEYWORDS: Syphilis, CLIA, ELISA, RPR, Blood donor screening

INTRODUCTION

Syphilis is a chronic systemic infection caused by *Treponema pallidum*, primarily transmitted through sexual contact and blood transfusion[1],[2]. Despite the availability of effective treatment, it remains a major global public health concern due to its diverse clinical manifestations and asymptomatic stages[1]–[3]. The disease is often referred to as the "great imitator," as it can mimic several other conditions, making laboratory diagnosis essential[3].

Screening of blood donors plays a crucial role in preventing transfusion-transmitted infections. Serological testing remains the cornerstone for diagnosis, particularly in asymptomatic individuals such as voluntary blood donors[4],[5]. These tests are broadly categorized into non-treponemal tests, such as Rapid Plasma Reagin (RPR), and treponemal tests, including Enzyme-Linked Immunosorbent Assay (ELISA) and Chemiluminescence Immunoassay (CLIA)[5].

RPR is widely used due to its simplicity and cost-effectiveness; however, its sensitivity varies depending on the stage of infection and may be reduced in early and late stages[6]. ELISA, a treponemal test, offers higher sensitivity and specificity and is capable of detecting both IgM and IgG antibodies[7].

Chemiluminescence Immunoassay (CLIA) is an advanced diagnostic method that provides enhanced sensitivity, automation, and high-throughput capability. It has been increasingly adopted in modern diagnostic laboratories and blood screening programs[7],[8]. Recent studies have demonstrated that CLIA can detect more reactive cases compared to conventional methods, supporting its role in reverse screening algorithms[8],[9].

Given the differences in diagnostic performance among these tests, comparative evaluation is essential to determine the most effective screening strategy. Therefore, this study was undertaken to compare the diagnostic performance of RPR and CLIA with ELISA among voluntary blood donors.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Blood Bank of a tertiary care hospital in Chennai from August 2024 to February 2026.

A total of 185 voluntary blood donors were included. Donors who consented were enrolled, while those unwilling or deferred were excluded.

Approximately 5 mL of blood was collected under aseptic conditions. Serum was separated and tested using:

- Rapid Plasma Reagin (RPR)

• Enzyme-Linked Immunosorbent Assay (ELISA)
• Chemiluminescence Immunoassay (CLIA)
These tests were performed according to standard protocols[5,10].
Statistical analysis was conducted using SPSS version 26. Chi-square test was used for association, and kappa statistics assessed agreement. A p-value < 0.05 was considered statistically significant.

OBSERVATION AND RESULTS
A total of 185 donors were selected between August 2024 and February 2026 to evaluate the test characteristics of Rapid Plasma Reagin card test and "Chemiluminescence immunoassay in comparison to Enzyme Linked Immunosorbent Assay in detection of syphilis among voluntary blood donors in the blood bank of tertiary care center**.**

Data Table 1: Distribution of Age

	Median	**Percentile 25**	**Percentile 75**
AGE	29	23	37

The median age of the participants was 29 years, indicating that half of the study population was younger than 29 and half was older.
The interquartile range, from 23 to 37 years, shows that the central 50% of participants were within this age group, suggesting a relatively young study population with moderate variability in age distribution.

Data Table 2 Distribution of Blood Group

		Count	**Column N %**
BLOOD GROUP	A NEGATIVE	2	1.1%
	A POSITIVE	43	23.2%
	AB POSITIVE	13	7.0%
	B NEGATIVE	3	1.6%
	B POSITIVE	63	34.1%
	O NEGATIVE	4	2.2%
	O POSITIVE	57	30.8%

B positive was the most common blood group among participants (34.1%), followed by O positive (30.8%) and A positive (23.2%). AB positive accounted for 7.0%, while negative blood groups were relatively less common, with O negative (2.2%), B negative (1.6%), and A negative (1.1%). Overall, Rh-positive blood groups predominated in the study population.

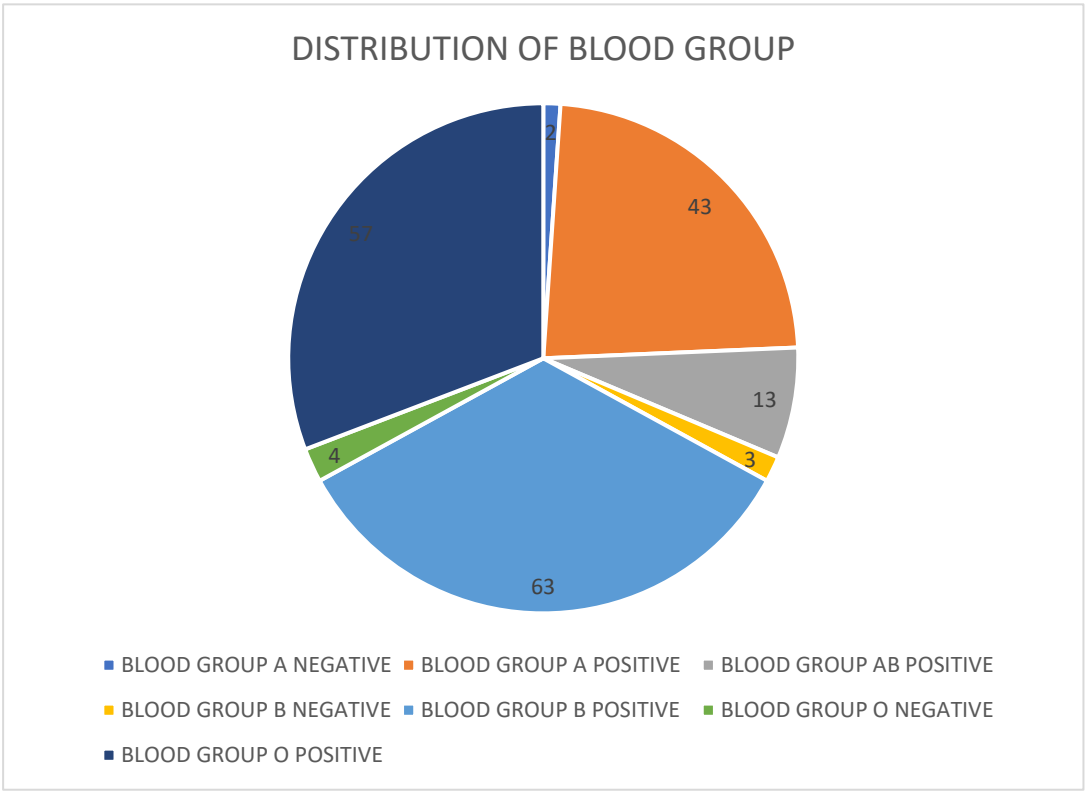

Chart 1: Distribution of Blood Group

Data Table 3: Association between CLIA And ELISA

		CLIA			
		NEGATIVE		POSITIVE	
		Count	Column N %	Count	Column N %
ELISA	NEGATIVE	146	94.8%	21	67.7%
	POSITIVE	8	5.2%	10	32.3%

Data Table 4: Chi-Square Tests

Chi-Square Tests			
	Value	df	P-VALUE
Pearson Chi-Square	21.519[a]	1	.000

Data Table 5: Measure of Agreement between CLIA and ELISA

		Value	P-VALUE
Measure of Agreement	Kappa	.325	.095

Among CLIA negative participants, the majority were ELISA negative (94.8%), while only 5.2% were ELISA positive. In contrast, among CLIA positive participants, 67.7% were ELISA negative and 32.3% were ELISA positive, indicating a higher proportion of ELISA positivity in this group.

The association between CLIA and ELISA was statistically highly significant ($p < 0.001$), suggesting a strong relationship between the two tests. However, the agreement between CLIA and ELISA was only fair (kappa = 0.325) and not statistically significant ($p = 0.095$), indicating limited concordance despite the significant association.

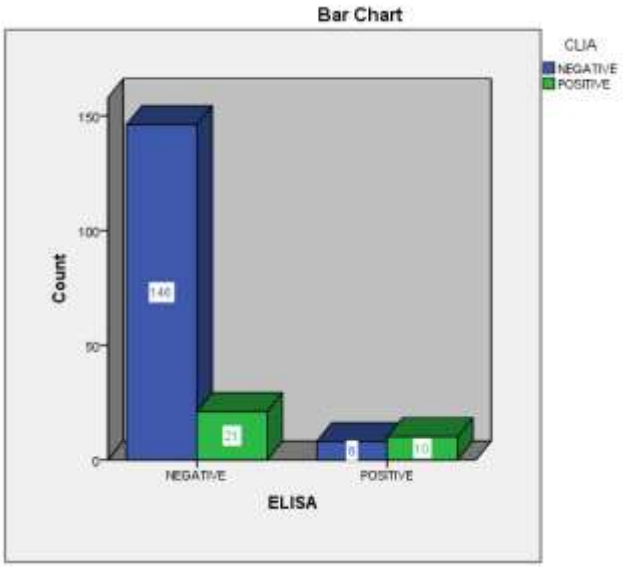

Chart 2: Association of ELISA with CLIA

Data Table 6: Association of ELISA With RPR

		RPR			
		NEGATIVE		POSITIVE	
		Count	Column N %	Count	Column N %
ELISA	NEGATIVE	165	94.8%	2	18.2%
	POSITIVE	9	5.2%	9	81.8%

Data Table 7: Fisher's Exact Test

	Value	df	P-VALUE
Fisher's Exact Test	69.199[a]	1	.000

Data Table 8: Measure of Agreement RPR and ELISA

		Value	P-VALUE
Measure of Agreement	Kappa	.590	.000

Among RPR negative participants, the majority were ELISA negative (94.8%), with only 5.2% being ELISA positive. In contrast, among RPR positive participants, 81.8% were ELISA positive and only 18.2% were ELISA negative, indicating a marked increase in ELISA positivity in this group.

The association between ELISA and RPR was statistically highly significant ($p < 0.001$), demonstrating a strong relationship between the two tests. Additionally, the agreement between ELISA and RPR was moderate (kappa = 0.590) and statistically significant ($p < 0.001$), indicating a good level of concordance between the tests.

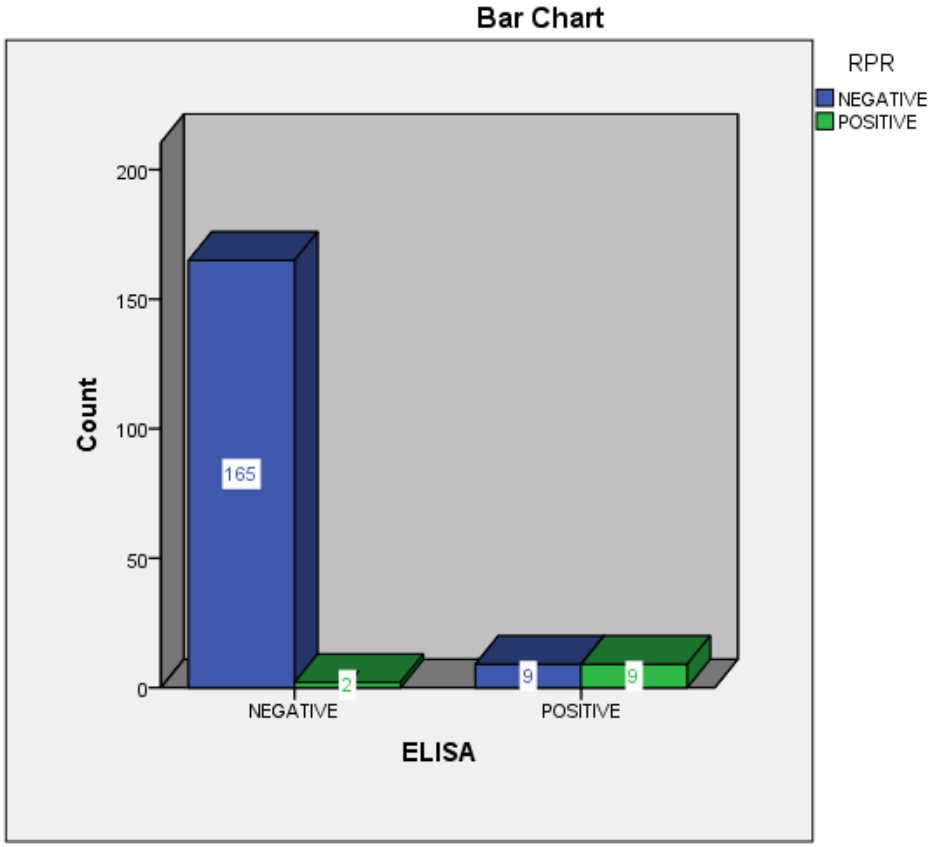

Chart 3: Association of ELISA with RPR

DISCUSSION

The present study was carried out to compare the performance of rapid diagnostic test and chemiluminescence immunoassay (CLIA) with enzyme linked immunosorbent assay (ELISA) for the diagnosis of syphilis among voluntary blood donors. ELISA was considered as the reference method because of its established role in screening blood donors and its higher sensitivity for detecting treponemal antibodies. The study also examined the association of test results with age, sex and blood group.

A total of 185 voluntary blood donors were included in the study. The median age of the participants was 29 years, with most donors belonging to the age group of 23–37 years. This indicates that the study population was relatively young.

The study population showed a marked male predominance. Out of 185 donors, 180 (97.3%) were males and only 5 (2.7%) were females. The most common blood group in the study population was B positive, followed by O positive and A positive. This pattern is similar to the blood group distribution commonly seen in the Indian population.

Distribution of Syphilis Positivity by Different Tests

The overall positivity rate varied depending on the test used. RPR showed positivity in 11 donors (5.9%), ELISA in 18 donors (9.7%) and CLIA in 31 donors (16.8%). The difference in positivity rates suggests that the three tests differ in their ability to detect syphilis infection.

The RPR test identified the lowest number of positive cases. RPR mainly detects antibodies produced in response to cellular damage caused by infection. Because of this, RPR may miss cases in the early stage, latent stage or previously treated cases of syphilis. Therefore, the lower positivity rate seen with RPR is expected.

ELISA detected more positive cases than RPR. ELISA detects antibodies specifically directed against *Treponema pallidum*. Since treponemal antibodies remain detectable for a longer period, ELISA can identify both current and past infection. This explains why ELISA positivity was higher than RPR positivity.

CLIA showed the highest positivity rate among all the tests. CLIA is considered more sensitive than ELISA because it uses chemiluminescent technology, which can detect even very low levels of antibodies. The higher number of positive cases by CLIA may therefore indicate its better ability to detect early or weakly positive infections. However, the higher positivity may also include false positive results, particularly in a low prevalence population such as voluntary blood donors.

These findings are similar to the studies of Morshed and Singh, who reported that treponemal assays (ELISA and CLIA)detect more positive cases than non-treponemal tests like RPR, and Fakile et al., who showed that reverse

screening with treponemal tests identifies additional cases missed by RPR [9,12]

Comparison of ELISA with RPR

One of the major objectives of this study was to compare rapid test performance with ELISA. The RPR test was used as the rapid screening test in this study.

Among the 174 donors who were RPR negative, 165 were also ELISA negative, while 9 were ELISA positive. This means that ELISA detected additional positive cases that were missed by RPR. Among the 11 RPR-positive donors, 9 were ELISA positive and 2 were ELISA negative.

These findings indicate that RPR may miss some cases of syphilis that can be identified by ELISA. The nine donors who were RPR negative but ELISA positive may represent latent, previously treated or very early syphilis cases in which non-treponemal antibodies are absent or present at a low level.

Therefore, RPR is good at confirming disease when the result is positive, but it may fail to detect some infected individuals. In blood donor screening, missing infected donors is dangerous because it can lead to transfusion-transmitted syphilis. Thus, RPR alone may not be sufficient as a screening test.

The results of the present study are similar to those of Kakkar et al., who reported that ELISA had better sensitivity than RPR for the detection of syphilis in blood donors. Negash et al. also observed that RPR showed lower sensitivity when compared with treponemal assays [11].

Comparison of CLIA with ELISA

The second major objective of this study was to compare CLIA with ELISA.

Among the 154 CLIA-negative donors, 146 were also ELISA negative and 8 were ELISA positive. Among the 31 CLIA-positive donors, only 10 were ELISA positive, while 21 were ELISA negative.

This means that CLIA detected many more positive cases than ELISA. However, a large proportion of CLIA-positive cases were ELISA negative. This suggest that

CLIA may detect weak antibody levels that are not identified by ELISA. Some of these weakly positive cases may represent early infection, past treated infection or biologically false positive reactions.

Several studies have shown that CLIA is highly sensitive for syphilis screening but may produce false positive results. Park et al. and Tong et al. reported that CLIA detected more reactive samples than ELISA, but confirmatory testing was needed to distinguish true positives from false positives [7,8].

Interpretation of Test Characteristics

The findings of the present study suggest that each test has its own advantages and limitations.

RPR showed good specificity and a high positive predictive value. Therefore, when the RPR test is positive, there is a high chance that the donor is truly infected. However, because its sensitivity is low, RPR may miss many infected donors.

Compared with RPR, ELISA identified more positive cases. Compared with CLIA, ELISA produced fewer false positive results.

CONCLUSION

The present study, conducted among voluntary blood donors, aimed to compare the diagnostic performance of rapid plasma reagin (RPR), enzyme-linked immunosorbent assay (ELISA), and chemiluminescence immunoassay (CLIA) for the detection of syphilis. Based on the statistical findings, CLIA demonstrated clear advantages over both RPR and ELISA.

In this study, CLIA identified a higher proportion of positive cases (16.8%) compared to ELISA (9.7%) and RPR (5.9%)

Although a statistically significant association was observed between CLIA and ELISA ($p < 0.001$), the agreement between the two tests was only fair (kappa = 0.325). This finding highlights that CLIA may detect more true positive cases that are not identified by ELISA, reinforcing its higher diagnostic yield. In contrast, ELISA showed only moderate agreement with RPR (kappa = 0.590), suggesting comparatively lower performance when used alone.

Furthermore, the ability of CLIA to function as an automated, high-throughput assay makes it particularly suitable for large-scale screening in blood banks and clinical laboratories. Its increased sensitivity, along with operational efficiency, provides a significant advantage over both ELISA and rapid tests.

In conclusion, CLIA emerged as the superior diagnostic modality in this study for the detection of syphilis among voluntary blood donors. It demonstrates higher sensitivity and better case detection compared to ELISA and RPR. Therefore, CLIA can be recommended as a reliable screening tool, either as a primary test or as part of a diagnostic algorithm, to enhance the accuracy and safety of blood donor screening programs.

REFERENCE LIST

1. Anteric I, Basic Z, Vilovic K, Kolic K, Andjelinovic S. Which theory for the origin of syphilis is true? J Sex Med. 2014;11(12):3112–8.
2. Lopez P, Kwentoh I, Valdez M. A peculiar presentation of syphilis. Cureus. 2023;15(9):e45328.
3. Peeling RW, Hook EW. The pathogenesis of syphilis: the great mimicker, revisited. J Pathol. 2006;208(2):224–32.
4. Zeltser R, Kurban AK. Syphilis. Clin Dermatol. 2004;22(6):461–8.
5. Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. Clin Microbiol Rev. 1995;8(1):1–21.

6. World Health Organization. Global prevalence and incidence of selected curable sexually transmitted infections. WHO; 2001.
7. Park IU, Chow JM, Bolan G, Stanley M, Shieh J. Performance of chemiluminescent immunoassays in the diagnosis of syphilis. Sex Transm Dis. 2011;38(7):620–4.
8. Tong ML, Lin LR, Liu LL, Zhang HL, Huang SJ, Chen YY, et al. Evaluation of chemiluminescence immunoassay for detection of Treponema pallidum antibodies. J Clin Lab Anal. 2017;31(3):e22060.
9. Fakile YF, Jost H, Hoover KW. Performance of treponemal tests in reverse screening algorithm. Clin Infect Dis. 2015;61(2):S86–S90.
10. Ratnam S. The laboratory diagnosis of syphilis. Can J Infect Dis Med Microbiol. 2005;16(1):45–51.
11. Negash M, et al. Seroprevalence of syphilis among blood donors: systematic review. BMC Infect Dis. 2018;18:551.
12. Morshed MG, Singh AE. Recent trends in the serologic diagnosis of syphilis. *Clin Vaccine Immunol*. 2015;22(2):137–147.