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Comparative evaluation of enzyme-linked immunosorbent assay with rapid plasma reagin for screening of syphilis in blood donors

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Abstract:

World Health Organization (WHO) recommends screening of syphilis in low prevalence populations of blood donors by treponemal tests like enzyme-linked immunosorbent assay (ELISA), whereas in India screening is done by rapid plasma reagin (RPR). The present pilot study evaluated the performance of ELISA compared to RPR, keeping *Treponema pallidum* hemagglutination assay as a reference test. ELISA was equally sensitive (100%), more specific (56.3% vs. 0%), more accurate (83.7% vs. 62.7%), had better positive predictive value (79.4% vs. 62.8%) and negative predictive value (100% vs. 0%), and less biological false positivity (37.2% vs. 20.6%) when compared to RPR. The WHO recommendations of screening for syphilis in low prevalence population of blood donors using ELISA may be adopted for usage in transfusion services that have the facility of ELISA.

Keywords:

Biological false positivity, enzyme-linked immunosorbent assay, negative predictive value, positive predictive value, rapid plasma reagin, syphilis, *Treponema pallidum* hemagglutination assay, Venereal Disease Research Laboratory

Introduction

In India, Drugs and Cosmetics Act of 1940 and the Rules of 1945 as amended from time to time, mandate screening for syphilis in blood donors by Venereal Disease Research Laboratory (VDRL) test.[1] The World Health Organisation (WHO) however, recommends screening of syphilis in blood donors by treponemal tests like enzyme linked immunosorbent assay (ELISA).[2] VDRL and rapid plasma reagin (RPR) are nontreponemal tests and detect antiphospholipid antibodies against the lipids released from damaged host cells and the lipid in the cell wall of treponeme.[2] These tests are simple to perform, are rapid, inexpensive and indicate active infection. But these tests have certain limitations. These tests are less specific and may show false negative (FN) results due to prozone phenomenon.[3,4] The print-outs of results are not available for documentation purpose and the ratio of VDRL antigen and test serum can vary especially while testing large number of samples in high throughput blood banks. Another problem with these tests is the phenomenon of biological false positivity (BFP).[3,4] Treponemal tests like ELISA detects human antibodies against *Treponema pallidum* and are mainly used as confirmatory tests to verify the reactivity seen in nontreponemal tests. These tests are more specific, provide objective printout of test results and the testing platform is amenable to automation.[3,4] ELISA test runs may be subjected to quality control by using in house and external controls. Calculating E

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ratios and plotting Levy-Jennings charts helps in tracking intra and inter assay variations.

Keeping in view the WHO recommendation for syphilis screening, this pilot study was conducted to evaluate the performance of ELISA as a screening tool for screening of syphilis in blood donors compared to RPR, keeping *T. pallidum* hemagglutination assay (TPHA) as reference test.

Materials and Methods

This study was conducted after taking approval from Institutional Ethical Committee of the institute. In phase 1 (one), we conducted simultaneous screening of 600 consecutive donor serum samples by ELISA and RPR, in order to evaluate the technical performance of ELISA at our centre. In phase 2, repository of 43 repeat reactive samples (on two RPR kits) was tested on ELISA kits at Department of Transfusion Medicine and Gastroenterology Virology (GE Virology) independently. TPHA was done at Department of Medical Microbiology. All those samples which initially came negative by TPHA were again repeated and reconfirmed. The study methodology was designed after reviewing the literature.[3-9] RPR test was performed using Carbogen (Tulip Diagnostics (P) Ltd., India) and RPR test (Span, Arkray Healthcare Private Limited, India). ELISA was done using ERBALISA SYPHILIS kits (Transasia Bio-medicals Ltd., India) which detect total (IgG, IgM, IgA etc.) antibodies using a mixture of recombinant Treponemal proteins coated on microwells. TPHA was done using IMMUTREP TPHA (Omega Diagnostics Limited, U.K). All tests were performed strictly as per manufacturer's instructions.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and the percentage of false positive (FP) results obtained by RPR and ELISA were calculated keeping TPHA as a reference test.

True positive (TP) is when the test results are reactive; if the disease is present i.e., TPHA is positive.

True negative (TN) is when the test results are nonreactive; if the disease is not present i.e., TPHA is negative.

FN is when the test results are nonreactive; if the disease is present i.e., TPHA is positive.

FP is when the test results are reactive; if the disease is not present i.e., TPHA is negative.

Sensitivity is defined as the probability that a test result will be positive when the disease is present (TP rate).

Sensitivity = TP/TP + FN

Specificity is defined as the probability that a test result will be negative when the disease is not present (TN rate).

Specificity = TN/TN + FP

PPV is defined as the probability that the disease is present when the test is positive.

PPV = TP/TP + FP

NPV is defined as the probability that the disease is not present when the test is negative.

NPV = TN/TN + FN

Accuracy = TP + TN/total number of samples

BFP = Total number of FP results/total positive results given by the test under evaluation

Sensitivity, specificity, positive and NPVs were expressed as percentages for ease of interpretation. Their confidence intervals (CI) were "exact" Clopper-Pearson CI. All the statistical analysis in this study was done using MedCalc online diagnostic software (MEDCALC®, Acacialaan 22, Ostend, Belgium).

Results

In phase 1, 600 consecutive donor serum samples which were nonreactive on RPR test were also nonreactive on ELISA.

In phase 2, 43 repeat reactive repository samples (reactive on two RPR kits) were screened by ELISA and confirmed by TPHA. On analysis TPHA gave the positive result in 27 serum samples while it was negative in 16 samples. ELISA gave reactive results on 34 samples and was nonreactive in 9 samples. The results are depicted in Table 1.

Based on the analysis of the above data; the sensitivity, specificity, PPV, NPV and accuracy of the RPR and ELISA were calculated keeping TPHA as a reference test,

Table 1: Comparison of results obtained on rapid plasma reagin and enzyme linked immunosorbant assay with *Treponema pallidum* haemagglutination assay

TPHA (*n*=43)	RPR		ELISA	
	Positive	Negative	Positive	Negative
Positive (27)	27 (TP)	0 (FN)	27 (TP)	0 (FN)
Negative (16)	16 (FP)	0 (TN)	7 (FP)	9 (TN)

TP=True positive, TN=True negative, FP = False positive, FN = False negative, TPHA = *Treponema pallidum* haemagglutination assay, RPR = Rapid plasma reagin, ELISA = Enzyme linked immunosorbant assay

Table 2: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of rapid plasma reagin and enzyme linked immunosorbant assay compared to *Treponema pallidum* haemagglutination assay

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (%)
RPR	100% (87.23%-100%)	0% (0.00%-20.59%)	62.79% (46.73%-77.02%)	0%	62.7
ELISA	100% (87.23%-100%)	56.25% (29.88%-80.25%)	79.41% (62.10%-91.30%)	100% (66.37%-100%)	83.72

PPV = Positive predictive value, NPV = Negative predictive value, RPR = Rapid plasma reagin test, ELISA = Enzyme linked immunosorbant assay, CI = Confidence interval

and the results are shown in Table 2. In the present study the sensitivity of the two methods was same, ELISA was more specific, had better PPV and NPV, and was more accurate when compared to RPR as a screening method. The results of BFP obtained with RPR and ELISA are shown in Table 3. It was observed that BFP was more with RPR as compared to ELISA.

The comparison of the various parameters assessed to evaluate ELISA and RPR with TPHA with other studies is represented in Table 4.

Optical density of each of the 43 repeat RPR reactive serum samples obtained by ELISA was divided by cut-off value and the signal to cut-off obtained was evaluated against the TPHA. receiver operating curve curves was applied to find out sensitivity, specificity, NPV, PPV (95% CI) for different values of signal to cut-off ratio. It was observed that the signal to cut-off ratio of 7.72 on ELISA, had a sensitivity of 100% (95% CI: 87.2%–100%), 93.75% specificity (95% CI: 69.8%–99.8%), PPV of 96.4% (95% CI: 81.7%–99.9%) and NPV of 100% (95% CI: 78.2%–100%) with Area under the curve of 99.3% (95% CI: 90.5%–100%) at $P = 0.0001$.

Discussion

No single test is ideal for diagnosing syphilis since each test has some limitations in terms of sensitivity, specificity and the results of a particular test varies depending upon the stage of syphilis. The sensitivity of RPR in the present study was found to be comparable with ELISA at 100% while specificity was found to be 0% for RPR and 56.2% for ELISA. Saral *et al*. have reported a lower sensitivity of 58%, and a comparable specificity of 0% of RPR compared to TPHA.[5] The 0% specificity of RPR obtained in the present study has to be interpreted keeping in view that this is obtained in a population of blood donors and thus may not be extrapolated to other clinical settings where in clinical suspicion is present and the test is ordered based on the clinical suspicion. Naidu *et al*. reported the sensitivity of RPR as 70% and specificity as 54.28%.[6] The PPV of ELISA in the present study was 79.4% is lower than 99.3% reported by Woznicová and Valisová.[9] Whereas the PPV of RPR in the present study was 62.8% which is lower than 92% reported by Saral *et al*.[5] The NPV of ELISA in the present study was 100% comparable with

Table 3: Biological false positivity with rapid plasma reagin and enzyme linked immunosorbant assay

Test	BFP	Percentage of BFP	OR (95% CI)	*P*
RPR	16	16/43 (37.20)	2.28 (0.81-6.44)	0.13
ELISA	7	7/34 (20.58)		

BFP = Biological false positivity, CI = Confidence interval, OR = Odds ratio, RPR = Rapid plasma reagin test, ELISA = Enzyme linked immunosorbant assay

97.2% reported by Woznicová and Valisová.[9] Further the 0% NPV of RPR obtained in the present study has earlier been also reported by Saral *et al*.[5] The accuracy of ELISA in the present study was 83.7% compared to 62.7% of RPR. Saral *et al*. have reported accuracy of 56% with RPR.[5] Previous studies by Young *et al*. using wild type of treponemal pallidum antigens reported the sensitivity of ELISA to be 98.4% and specificity of 99.3% compared to TPHA and fluorescent treponemal antibody absorption.[10] Young *et al*. while using recombinant antigen of treponema reported sensitivity of ELISA to be 99% and specificity of 91.4%.[11] A comparative evaluation of ten different enzyme immunoassay using wild or recombinant antigens by Cole *et al*. the sensitivity of ELISA was found to vary between 94.7%-99.1% while specificity was 100%.[12]

The BFP obtained in the present study is more with RPR (37.2%) compared to ELISA (20.5%) with odds of 2.28 (0.81–6.44) at $P = 0.13$, which is much less than 56.4 % for RPR and 50% for ELISA reported by Naidu *et al*. from Mumbai, India.[6] BFP (short-term or long-term persistence) has been attributed to strong immunological stimulus like viral infections, systemic lupus erythematosis, thyroiditis, rheumatoid arthritis, atopic dermatitis, vaccination, or may occur even in apparently healthy people with advancing age.[3,13,14]

Importantly the characteristic of treponemal tests like ELISA of test being reactive despite treatment could be utilized to significant advantage in blood donor screening as a surrogate marker of high risk behavior of the blood donor at any point of time during the lifetime. This is not the case when using nontreponemal tests like VDRL/RPR, which usually turn negative after successful treatment. Therefore a negative treponemal screening test could help blood bank to select a low risk donor. Further keeping in view that for blood donors screening a test of high sensitivity and high specificity is recommended, the preliminary results of the present study of very low specificity of RPR do not support the usage of RPR in

Table 4: Comparison of enzyme linked immunosorbant assay and rapid plasma reagin in different studies

	Percentage											
	Sensitivity		Specificity		PPV		NPV		Accuracy		BFP	
	RPR	ELISA	RPR	ELISA	RPR	ELISA	RPR	ELISA	RPR	ELISA	RPR	ELISA
Present study	100	100[#]	0	56.2[#]	62.8	79.4	0	100	62.7	83.7	37.2	20.5
Naidu *et al*.	70	96.2	54.2	50	-	-	-	-	-	-	56.4	50
Vladana *et al*.	-	99	-	98	-	99.3	-	97.2	-	-	-	-
Young *et al*.	-	98.4* and 99[#]	-	99.3* and 91.4[#]	-	-	-	-	-	-	-	-
Cole *et al*.	-	94.7-99.1[$]	-	100[$]	-	-	-	-	-	-	-	-
Saral *et al*.	58	-	0	-	92	-	0	-	56	-	-	-

*Wild antigen, [#]Recombinant antigen, [$]Wild or recombinant antigen. PPV = Positive predictive value, NPV = Negative predictive value, RPR = Rapid plasma reagin test, ELISA = Enzyme linked immunosorbant assay, BFP = Biological false positivity

blood donor setting. It may be reemphasized that quality control practices can be put in place by using ELISA and this will ensure compliance with accreditation and good manufacturing and laboratory practices.

In the present study, at a signal to cut-off ratio value of 7.72 or above on ELISA, it was found that the possibility of the test result was more likely to be TP, though this value may be limited by the main limitation of the present pilot study in terms of sample size. The other limitation of the present study was the inability to detect FNs on RPR testing because the study utilized a repository of stored repeat reactive RPR samples. Further the specificity obtained for RPR in the study could be biased by the inclusion of samples from apparently healthy blood donors without and clinical symptomatology. However the results support the need to evaluate the efficacy of ELISA as a screening tool in blood donors on a large scale preferably including different geographical regions.

Conclusion

ELISA was equally sensitive (100%), more specific (56.3% vs. 0%), more accurate (83.7% vs. 62.7%), had better PPV (79.4% vs. 62.8%) and NPV (100% vs. 0%), and less BFP (37.2% vs. 20.6%) when compared to RPR in the present evaluation. The WHO recommendations of screening for syphilis in low prevalence population of blood donors using ELISA may be adopted for usage in transfusion services that have facility of ELISA.

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Conflicts of interest

There are no conflicts of interest.

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