Original Article

Evaluation of a new serological test for syphilis based on chemiluminescence assay in a tertiary care hospital

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Abstract:

Context: Syphilis is a transfusion transmissible infections and it is mandatory to do serological test for syphilis (STS) on all donor blood samples. STS is usually based on detection of antibodies against the cardiolipin-lecithin antigen or against the *Treponema*-specific antigen. STS with good sensitivity and specificity helps enhance blood safety and consolidation of STS along with other transfusion transmittable infections such as human immunodeficiency virus, hepatitis-C virus, and hepatitis-B virus helps in reducing the errors and enhances efficiency. **Aims:** This study was designed to evaluate the performance of newly introduced VITROS® syphilis *Treponema pallidum* agglutination (TPA) assay based on enhanced chemiluminescence principle for its analytical performance for use as a STS on donor blood samples at a tertiary care health center in National Capital Region, India. **Materials and Methods:** A total of 108 random blood units collected from the donors (both voluntary and replacement donors) and 28 known syphilis sero-reactive samples stored at −20°C, were used to evaluate the performance of VITROS® syphilis TPA assay based on enhanced chemiluminescence assay on VITROS® ECiQ immunodiagnostics system along with its analytical performance in terms of its sensitivity, precision, cross-reactivity and interference studies. **Results:** VITROS® syphilis TPA showed 100% sensitivity and specificity with precision (20 days study) of <10% co-efficient of variation. There was no cross-reactivity with other viral and auto-immune antibodies. No interference was observed from endogenous interfering substances like free hemoglobin or fats. **Conclusions:** Performance of the VITROS® syphilis TPA assay meets the requirements for its use as STS in blood bank, thus allowing consolidation with other transfusion transmittable infections screening assay on chemiluminescence platform, which is highly valuable for optimizing workflow and efficiency.

Key words:

Non-treponemal test, serological test for syphilis, treponemal test

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Introduction

Syphilis is a sexually transmitted disease caused by the spirochetal bacterium *Treponema pallidum* (TP). The route of transmission of syphilis is almost always sexual contact. The infection may also be passed congenitally from mother to the unborn child causing birth defects or fetal death. Rarely, the infection may also get transmitted through blood transfusion.

Diagnosis of syphilis is currently based on several criteria: Patient history, clinical symptoms, serological tests, and identification of TP in lesions or tissue. Direct detection of treponemes or treponemal antigen requires special skills or use of complex reagents with short shelf life. Thus, the serological test for syphilis (STS) acts as a better alternative for laboratory-diagnosis. STS is usually based on detection of antibodies against the cardiolipin-lecithin antigen (nontreponemal) or against the *Treponema*-specific antigen. Routinely nontreponemal tests such as venereal disease research laboratory or rapid plasma reagin (RPR) tests are commonly used for donor blood screening.[1,2] However, large number of false positive results and low sensitivity of these tests have led to the introduction of TP-specific tests for syphilis screening. The most commonly used TP-specific assays for laboratory-diagnosis are the TP hemagglutination (TPHA) test and the fluorescent treponemal antibody-absorption test.[3]

Recently, enhanced chemiluminescence assay using recombinant TP antigen for the detection of both IgM and IgG specific antibodies for screening syphilis infection has been introduced. Enhanced chemiluminescence assay is known for its excellent sensitivity and specificity for the infectious disease screening. The objective of this study was to evaluate the analytical performance of VITROS® syphilis TP agglutination (TPA) assay in screening healthy blood donors for syphilis infection based on the qualitative detection of antibodies against TP antigen in VITROS® ECiQ immunodiagnostic system.

Materials and Methods

The study was conducted in the Department of Transfusion Medicine, in a tertiary care hospital

in National Capital Region, India with three different lots of VITROS® syphilis TPA Reagent Pack and the VITROS® syphilis TPA calibrator on the VITROS® ECi/ECiQ immunodiagnostic system. VITROS® syphilis TPA assay was evaluated for its analytical performance by processing healthy donor samples that were sero-negative for syphilis and stored sero-reactive donor samples.

A total of about 108 random blood samples were collected from healthy donors who had donated blood in the blood bank. All the donors were selected following strict donor selection criteria; administering medical history questionnaire and clinical examination. 28 known syphilis sero-reactive serum samples, identified earlier during routine screening, were stored at –20°C, These sero-reactive samples were also used in this study.

Three different lots of VITROS® syphilis TPA assay were calibrated in VITROS® ECiQ immunodiagnostics system using lot specific calibrator following manufacturer's instructions. The success of the calibration was verified by testing both VITROS® syphilis TPA negative and positive controls in duplicate.

Diagnostic accuracy

Both syphilis sero-negative and sero-reactive samples were tested with VITROS® syphilis TPA assay in three different lots in VITROS® ECiQ based on the manufacturer's instructions. All the samples were tested in parallel using syphilis immunochromatographic assay (SD Bioline syphilis 3.0 assay) as per manufacturer's instructions. The samples, which showed discordant results between VITROS® syphilis TPA assay and syphilis immunochromatographic assay were retested in syphilis TPHA assay (Plasmatec, Lab21 Healthcare Ltd., UK) as per manufacturer's instructions.

Inter-assay precision

Precision was evaluated on three different concentrations of anti-syphilis antibody, close to the cut-off limit based on the National Committee for Clinical Laboratory Standards (NCCLS) (Clinical and Laboratory Standards Institute [CLSI]) document EP5-A2.[4] Inter-assay precision was evaluated on three different concentrations close to the cut-off limit of which one level was below the cut-off limit (<1.0) and the other two levels were above the cut-off limit. A pool of human plasma from healthy donors was prepared and screened for the presence of any infectious disease markers such as hepatitis B surface antigen (HBsAg), anti-human immunodeficiency virus (HIV) antibody, anti-hepatitis-C virus (HCV) antibody and anti-treponemal antibody. In the pooled plasma, negative for all infectious disease markers, anti-treponemal antibody reactive sample was mixed at three different concentrations, aliquoted and stored at –20°C until used. Testing of all the three different samples was performed over 20 days, twice daily in VITROS® ECiQ immunodiagnostic system. For each level of control samples, mean, standard deviation (SD) and co-efficient of variation (CV) was calculated. The assessment criteria was CV% of <10% for all the three different concentrations.

Cross-reactivity

Cross-reactivity study was carried out to verify any cross-reaction of other viral antibody or auto-antibody in the VITROS® syphilis TPA assay. Samples, which are reactive for anti-HIV antibody, anti-HCV antibody, anti-cytomegalovirus (CMV) (IgG) antibody, HBsAg and samples from auto-immune disorders viz., rheumatoid diseases, systemic lupus erythematous (SLE), were tested in VITROS® syphilis TPA assay.

Interference

Interferences by endogenous substances such as hemolytic, icteric and lipemic samples were evaluated by diluting a pool of syphilis sero-reactive human sera with the samples having potentially interfering substances at two different concentrations, 1:1 and 1:3 dilution and tested in VITROS® syphilis TPA assay. The obtained results were verified for any interference when compared with the control sample without any interfering substances.

Dilution sensitivity

The detection limit at lower concentration of syphilis antibody was evaluated by serial dilution of the syphilis sero-reactive sample in syphilis nonreactive sample and tested simultaneously in both VITROS® syphilis TPA assay and syphilis immunochromatographic assay.

Results

Three different lots of VITROS® syphilis TPA assay were calibrated in VITROS® ECiQ immunodiagnostics system and verified using both VITROS® syphilis TPA negative and positive controls. Both controls were within the manufacturer's specification, showed signal/cut-off ratio of <0.1 for negative control and 2.9 ± 0.5 for positive control.

Diagnostic accuracy

Diagnostic accuracy was evaluated using a protocol based on NCCLS (CLSI) document EP9-A2. syphilis sero-negative and sero-reactive serum samples were tested for syphilis antibody using VITROS syphilis TPA assay, which is based on double antigen sandwich assay and one step syphilis anti-TP assay based on solid phase immunochromatograpic assay (SD Bioline syphilis 3.0). Twenty-five samples showed "reactive" results and 108 samples showed "nonreactive" results in both VITROS syphilis TPA of all the three different lots and syphilis immunochromatographic assay. Three samples showed discordant results, which were "reactive" in syphilis immunochromatographic assay and "nonreactive" in all the three different lots of VITROS syphilis TPA assay. All the three discordant samples were retested in syphilis TPHA assay and all these three samples were found to be negative in syphilis TPHA assay [Table 1]. Based on the above data, after resolution of three discordant samples, the sensitivity and specificity of VITROS syphilis assay was found to be 100%, whereas syphilis immunochromatographic assay showed sensitivity of 100% and specificity of 97% [Figure 1]. There was no lot-to-lot variation observed in VITROS syphilis TPA.

Inter-assay precision

Mean S/CO value for all three levels were 0.67, 2.09 and 3.07. SD was 0.04, 0.11 and 0.21, respectively. The precision was excellent at all the three levels and the CV% obtained was 6.08; 5.08, and 5.45 [Table 2]. The assessment criteria of CV% <10% were satisfied at all the three different concentrations evaluated.

Cross-reactivity

Cross-reactivity study was carried out to verify any cross-reaction in VITROS syphilis assay with any other antibody against viral antigens or auto-antibodies. Three samples each of anti-HIV

antibody, anti-HCV antibody, HBsAg, CMV (IgG) and rheumatoid factor and one sample for SLE were tested in VITROS® syphilis TPA assay. All samples were nonreactive and no cross-reactivity was observed [Table 3].

Interferences

Interferences by endogenous substances viz., hemoglobin, bilirubin, and lipids were evaluated by diluting the syphilis reactive samples with hemolyzed, icteric and lipemic samples at the concentration of 1:1 dilution and 1:3 dilution and tested in VITROS syphilis TPA assay in triplicate. The obtained results [Table 4] showed that there was no interference in VITROS syphilis TPA assay by the endogenous substances viz., hemolysed, icteric and lipemic samples and the obtained results were comparable with the control sample.

Dilution sensitivity

Dilution sensitivity study was carried out to compare the minimum detection limit of both VITROS syphilis TPA assay and syphilis immunochromatography assay by doing serial dilution of syphilis "reactive" sample and tested in both systems. In dilution sensitivity study [Table 5], VITROS syphilis assay showed "reactivity" up to 1:32 dilution, whereas syphilis immunochromatography assay showed "Reactivity" up to 1:4 dilution only.

Discussion

The VITROS ECiQ immunodiagnostic system is simple to use, fully automated laboratory test system. Our study evaluated VITROS syphilis TPA assay in 108 random blood samples and 28 sero-reactive serum samples collected from apparently healthy blood donors with simultaneous testing on solid phase immunochromatograpic assay. Three samples had discordant results which were confirmed by testing with another specific treponemal serologic test [Table 1]. In the present study, the VITROS syphilis TPA assay based on enhanced chemiluminescence principle performed very well as a screening test for healthy blood donors showing the relative sensitivity and specificity as 100% compared with immunochromatographic assay as 100% and 97% respectively. VITROS syphilis TPA assay precision was excellent at all the three levels [Table 2]. Cross-reactivity and interference was not observed with viral and auto-immune antibodies and other interfering endogenous substances. Lot-to-lot consistency was also there among all the three lots studied. This study showed that VITROS syphilis assay has an excellent dilutional sensitivity which was at least 8 times higher than syphilis immunochromatography assay [Table 5].

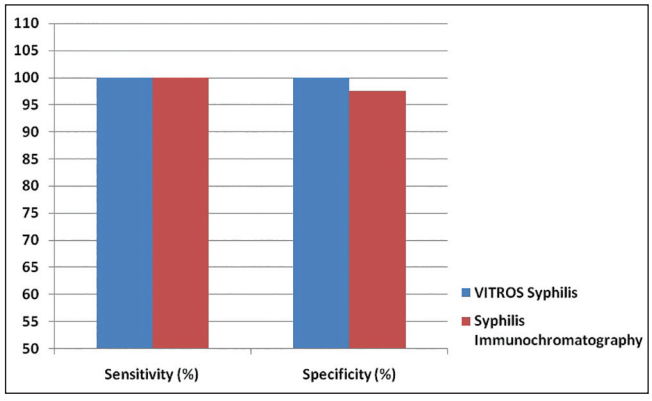

Figure 1: The sensitivity and specificity of VITROS® syphilis *Treponema pallidum* agglutination assay and syphilis immunochromatography assay based on sample comparison data

Incidence of transfusion transmitted syphilis has decreased in the recent years due to effective donor screening for high risk behavior, mandatory serological screening, storage of blood in refrigerator at 2-8°C and use of antibiotics. Overall, sero-prevalence of syphilis as per the published data in India is 0.7%[5-9] [Table 6]. There is no uniform testing method for screening of syphilis in blood donors in India. Most of the blood centers carry out the screening for syphilis using RPR method (nontrepenomal). Main advantage to this test is

Table 1: Diagnostic accuracy

Type of Assay		Syphilis IC		Total
		Reactive	Nonreactive	
VITROS syphilis	Reactive	25	0	25
TPA assay –	Nonreactive	3	108	111
Total		28	108	136

IC: Immunochromatography

Table 2: Inter-assay precision report of VITROS® syphilis TPA

Level	*n*	Mean (S/Co)	SD	CV%
1	40	0.67	0.04	6.08
2	40	2.09	0.11	5.08
3	40	3.77	0.21	5.45

SD: Standard deviation, TPA: *Treponema pallidum* agglutination, CV: Co-efficient of variation

Table 3: Cross-reactivity study report of VITROS® syphilis TPA

Reactive samples	Number	Result	Inference
Anti-HIV	3	Nonreactive	No cross-reactivity
Anti-HCV	3	Nonreactive	No cross-reactivity
HBsAg	3	Nonreactive	No cross-reactivity
CMV (IgG)	3	Nonreactive	No cross-reactivity
Rhematoid factor	3	Nonreactive	No cross-reactivity
SLE	1	Nonreactive	No cross-reactivity

TPA: *Treponema pallidum* agglutination, HIV: Human immunodeficiency virus, HCV: Hepatitis-C virus, HBsAg: Hepatitis B surface antigen, CMV: Cytomegalovirus, SLE: Systemic lupus erythematous

Table 4: Interference study report of VITROS® syphilis TPA

Interference samples	Dilution (1:1)	Dilution (1:3)
Hemolysed sample	2.08	0.96
Icteric sample	2.05	0.89
Lipemic sample	1.96	0.84
Normal sample (control)	2.09	0.98

TPA: *Treponema pallidum* agglutination

Table 5: Dilution sensitivity report of VITROS® syphilis TPA

Sample	VITROS® syphilis TPA	Syphilis IC
Neat	Reactive	Reactive
1:2 dilution	Reactive	Reactive
1:4 dilution	Reactive	Reactive
1:8 dilution	Reactive	Nonreactive
1:16 dilution	Reactive	Nonreactive
1:32 dilution	Reactive	Nonreactive
1:64 dilution	Nonreactive	Nonreactive

TPA: *Treponema pallidum* agglutination, IC: Immunochromatography

that it is inexpensive and simple to perform. Nontrepenomal tests are based on detection of antibodies against cardiolipin antigen and they require treponemal-based confirmation as antibodies against cardiolipin antigen can be produced by other conditions like viral infections, pregnancy, malignant neoplasms, auto-immune diseases, and advanced age.

Early syphilis is missed easily by serologic tests, as antibodies against the trepenomal antigen usually do not appear until 1-4 weeks of infection giving the false-negative results, a known limitation of all serologic tests. Sensitivity to detect antibodies against treponemal antigen varies according to the type of test and stage of infection[10-14] [Table 7], with lower sensitivities in primary syphilis and late syphilis. The advantage of the chemiluminescence assay studied is its high sensitivity in early syphilis due to its qualitative detection of antibodies both IgG and IgM and consolidation with other immunologic assays, which results in better workflow and enhanced efficiency. The EciQ can also handle high throughputs, which is otherwise very difficult with manual immunochromatography or RPR method. However, like other treponemal tests, it also cannot distinguish among recent, past and previously treated infections.

One of the major advantages of this assay is that it is available on an automated platform. Blood banks with high work-load may benefit from use of an automated screening test on a platform, which is already performing other TTI tests such as anti-HIV, anti-HCV and HBsAg. Automated treponemal based immunoassay may be an efficient screening test depending on the number of tests performed. Use of an automated assay also decreases the amount of technical time required.[15,16] Reisner *et al.* also observed that technical time required to perform an EIA screening procedure (automated immunoassay) was approximately half than that necessary for the manual RPR test.[16] The automated platform and consolidation with other immunoassay testing (HBsAg, anti-HIV and anti-HCV) improves operational efficiency providing rapid results allowing streamlined workflows and optimal blood release with an easy documentation and traceability.

Several other chemiluminescence based assays are also available, which detects both IgG and IgM and have been reported by various studies to be very sensitive treponemal tests with a very high specificity like LIASON assay by DiaSorin and Architect assay by Abbott. These assays have 95.8 and 98.4% sensitivity and 99.1% and 99.1% specificity, respectively.[14,17] In our study, we observed VITROS syphilis TPA showed the both relative sensitivity and specificity as 100%. The sample size in our study was smaller when compared with other studies and this was a limitation of our study. In our study, we also could not demonstrate the assay's ability to detect both IgG and IgM though the kit is capable of detecting both as per the kit insert.[18]

VITROS ECiQ immunodiagnostic system is a random access, fully automated analyzer and the technology is based on enhanced chemiluminescence technology, which helps in enhancing the sensitivity of the screening assay with the short turn-around-time. In reality, highly sensitive assay has a low chance of producing any false negative results. Based on the above study with three different lots of VITROS syphilis TPA assay on VITROS ECiQ immunodiagnostic system, it meets the requirements for its use as a screening assay for syphilis antibodies in blood donor serum or plasma samples to enhance the safety of the blood for transfusion.

Table 6: Sero-prevalence of syphilis in different parts of India

Place (reference)	Prevalence (%)
Haryana[5]	0.92
Lucknow[6]	0.01
West Bengal[7]	0.7
Karnataka[8]	1.6
Ludhiana[9]	0.085
Our center	0.8

Table 7: Sensitivity and specificity of STS

Tests (reference)	Sensitivity (%)				Specificity (%)
	Primary	Secondary	Latent	Late	
Nontrepenomal tests					
VDRL[10]	78	100	96	71	98
RPR[10]	86	100	98	73	98
Treponemal tests					
TPHA[11]	86	100	100	99	96
FTA-ABS[10]	84	100	100	96	97
ELISA based assays					
IgG ELISA[12]	100	100	100	—	100
IgM ELISA[13]	93	85	64	—	—
Chemiluminescence assay					
CLIA[14]	98	100	100	100	99

STS: Serological test for syphilis, VDRL: Venereal disease research laboratory, RPR: Rapid plasma regain, TPHA: *Treponema pallidum* hemagglutination assay, FTA-ABS: Fluorescent treponemal antibody-absorption, ELISA: Enzyme-linked immunosorbent assay, CLIA: Chemiluminescence immunoassay

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