Evaluation of an automated chemiluminescence assay for detection of syphilis infection from blood donors at 108 Military Central Hospital

Le Huy Khoi*, Do Giang Binh*, Nguyen Xuan Thanh*, Vu Hoang Trung, Pham Thi Thu Huong*, Vu Xuan Nghia***

**108 Military Central Hospital, **Thai Nguyen University of Medicine and Pharmacy*

Summary

Objective: The aim of this study was to evaluate VITROS(®) syphilis Treponema pallidum agglutination (VITROS(®) syphilis TPA) assay based on enhanced chemiluminescence principle for detection of syphilis infection in blood donors. *Subject and method:* A total of 110 random blood samples collected from the blood donors and 30 syphilis known 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(®) immunodiagnostics system to analyze the terms of its sensitivity, precision, cross-reactivity and interference studies. *Result*: The results showed that VITROS(®) syphilis TPA got 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. *Conclusion:* The results suggested that VITROS(®) syphilis TPA should be done to further reduce the risk of transfusion-transmitted TP infection, decrease unnecessary blood waste and loss of blood donors.

Keywords: Blood donor, syphilis, VITROS(®) syphilis TPA.

1. Background

Syphilis is a sexually transmitted bacterial disease caused by the spirochete Treponema pallidum. The infection may be passed congenitally from mother to unborn child, causing birth defects or fetal death [1]. Symptoms may come and go but the disease can remain latent and symptomless for years. If diagnosed early, syphilis can be easily treated with antibiotics, however, if it is not treated, syphilis can progress to a more dangerous form of the disease (tertiary syphilis) causing serious conditions such as stroke, paralysis, blindness or even death [2]. Serologic tests are still the mainstay for the diagnosis of syphilis and can be divided into nontreponemal and treponemal assays. Nontreponemal assays can be used to differentiate between active disease and past infection, which is not true for treponemal tests because these tests show a lifelong positivity. Because traditional treponemal tests are labor-intensive, newly introduced automated enzymatic immunoassays (EIAs) and chemiluminescence assays (CLIAs) offer a solution to the increasing number of samples sent to the laboratory.

Received: *19 October 2021,* ***Accepted:*** *25 November 2021* ***Correspondence to:*** *Vu Xuan Nghia - Department of Blood Transfusion, 108 Military Central Hospital*
Email: *nghia69@gmail.com*

Traditional algorithms for syphilis diagnostics start with a nontreponemal screening test (e.g., rapid plasma regain, or RPR), and when this test shows reactivity, confirmation is established with a treponemal test (e.g., T. pallidum particle agglutination, or TPPA). Such algorithms are thought to be cost-effective for small laboratories. Upon the arrival of automated immunoassays, a reverse algorithm was introduced that makes use of an EIA or CLIA as the primary screening test followed by a nontreponemal test only when the former is positive. If the nontreponemal assay turns out to be negative in a patient without a history of syphilis treatment, a second treponemal test, like the TPPA, is advised. Problems in interpretation may occur in case of discordant results, notably the combination of positive CLIA with negative RPR and TPPA results [3-5].

Syphilis has been detected in blood donors, mainly among them who have no symptoms. Currently, at the Department of blood transfusion, all samples are tested from blood donors attending the HIV, HBV, HCV and anti-TP by traditional assay. The TPHA has been used for screening syphilis. In order to increased test volumes, an automated system, the TPA assay (Ortho-Clinical Diagnostics, Rochester, NY) performed on Vitros 3600 Integrated System (Ortho-Clinical Diagnostics) was introduced. The purpose of this study was to evaluate the diagnostic performance of VITROS(®) syphilis TPA.

2. Subject and method

2.1. Specimens

A total of 110 random blood samples were collected from healthy. All the donors were selected following strict donor selection criteria; 30 known syphilis sero-reactive serum samples, identified earlier during routine screening, were stored at -20°C. The study was carry out in the Department of Blood Transfusion, in 108 Military Central Hospital.

2.2. Anti-TP screening by VITROS(®) syphilis TPA

The collected plasma samples were tested with an automated system, the TPA assay (Ortho-Clinical Diagnostics, Rochester, NY) performed on Vitros 3600 Integrated System (Ortho-Clinical Diagnostics). All procedures were carried out according to manufacturer's instruction. Testing results were expressed as signal to cutoff (S/CO), and ≤ 0.80 negative; ≥ 0.80 and < 1.20 borderline; ≥ 1.20 reactive. The sensitivity and the specificity of this assay are 100% and 99.76% respectively according to manufacturer's instruction.

Three different lots of VITROS® syphilis TPA assay were calibrated in VITROS® 3600 Integrated 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.

2.3. Analytical performance in terms of study

Diagnostic accuracy

Both syphilis sero-negative and sero-reactive samples underwent tested with VITROS® syphilis TPA assay in three different lots in Vitros 3600 Integrated System based on the manufacturer's instructions. All the samples were tested in parallel using syphilis immunochromatographic assay (SD Bioline syphilis 3.0 assay) according to manufacturer's instructions. If the results of samples 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 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. All human plasma healthy donors were screened for the presence of other pathogens 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 other pathogen 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 3600 Integrated System. For each level of control samples, mean, standard deviation (SD) and co-efficient of variation (CV) was calculated. The assessment criteria were CV% of < 10% for all the three different concentrations.

The cross-reactivity study was done to verify any cross-reaction of other viral antibody or auto-antibody in the VITROS® syphilis TPA assay. All 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, were tested in VITROS® syphilis TPA assay.

Interferences also were done 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 dilutions and tested in VITROS® syphilis TPA assay. The results were verified for any interference when compared with the control sample without any interfering substances.

Dilution sensitivity. In the case of the detection limit at lower concentration of syphilis antibody, it 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.

3. Result

Both VITROS® syphilis TPA negative and positive controls were verified and calibrated in Vitros 3600 Integrated System by three different lots of VITROS® syphilis TPA assay. 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.

3.1. Diagnostic accuracy

Table 1. The results of VITROS syphilis TPA and syphilis IC

Type of assay		*Syphilis IC*		Total
		Reactive	Non.Re	
VITROS syphilis TPA	Reactive	26	0	26
	Non reactive	4	110	114
Total		30	110	140

The data above showed that 26 samples were "reactive" and 110 samples "nonreactive" in both VITROS® syphilis TPA of all the three different lots and syphilis immunochromatographic assay (Syphilis IC). 4 samples were discordant results, which were "nonreactive" in all the three different lots of VITROS® syphilis TPA assay and "reactive" in syphilis IC assay. The four discordant samples were retested in syphilis TPHA assay and all these samples were found to be negative in syphilis TPHA assay. Based on the data, the sensitivity and specificity of VITROS® syphilis TPA assay was found to be 100%, whereas syphilis IC assay showed sensitivity of 100% and specificity of 96.5%. There was no lot-to-lot variation observed in VITROS® syphilis TPA.

3.2. Inter-assay precision

Table 2. The results of inter-assy of VITROS® syphilis TPA assay

Conc.	n	Mean (S/CO)	SD	CV%
1	40	0.61	0.03	6.05
2	40	2.12	0.12	5.03
3	40	3.09	0.22	5.47

The results showed that mean S/CO value for three concentrations were 0.61, 2.12 and 3.09. SD result was 0.03, 0.12 and 0.22, respectively. The precision was excellent at all the three concentrations and 6.05, 5.03, and 5.47 were obtained for CV%. It also presented the assessment criteria of CV% < 10% that satisfied at all the three different concentrations evaluated.

3.3. Cross-reactive results

Table 3. The results of cross-reactive by VITROS® syphilis TPA assay

Pathogens Samples	n	Results	Infernce
Anti-HIV	2	Non.re	No.cross
Anti-HCV	2	Non.re	No.cross
HBsAg	2	Non.re	No.cross
CMV (IgG)	2	Non.re	No.cross
Rheumatoid factor	1	Non.re	No.cross

Two samples of each other pathogens and one auto-antibody were evaluated for cross-reactive by VITROS® syphilis TPA. All samples were nonreactive and no cross-reactivity.

3.4. Interference of VITROS syphilis TPA assay

Table 4. The results of interference by VITROS® syphilis TPA assay

Samples	Dilution (1:1)	Dilution (1:3)
Hemolysed samples	2.05	0.94
Icteric sample	2.0	0.91
Lipermic sample	1.98	0.83
Normal sample (control)	2.06	0.97

All samples were diluted at the concentration 1:1; 1:3 and tested in VITROS® syphilis TPA assay in triplicate. The results showed that there was no interference in VITROS® syphilis TPA assay and the results also were compared with the control sample.

3.5. Dilution sensitivity of VITROS® syphilis TPA assay

Table 5. The results of Dilution sensitivity by VITROS® syphilis TPA assay

Sample dilution	VITROS syphilis TPA assay	Syphilis IC
Non dilution	Reactive	Reactive
Dilution 1:2	Reactive	Reactive
Dilution 1:4	Reactive	Reactive
Dilution 1:8	Reactive	Non.reactive
Dilution 1:16	Reactive	Non.reactive
Dilution 1:32	Reactive	Non.reactive
Dilution 1:64	Non.reactive	Non.reactive

The serial dilution of syphilis "reactive" sample was carried out to compare the minimum detection limit of both VITROS® syphilis TPA assay and syphilis IC assay by tested in both systems. The results in

table 5 showed that VITROS® syphilis TPA assay showed "reactivity" up to 1:32 dilution, whereas syphilis IC assay showed "Reactivity" up to 1:4 dilutions only.

4. Discussion

VITROS® syphilis TPA assay was studied to screen in 110 random healthy blood donor samples and 30 sero-reactive serum samples collected from apparently healthy blood donors with simultaneous testing on solid phase immunochromatograpic assay. Four samples had discordant results which were confirmed by testing with another specific treponemal serologic test. The VITROS® syphilis TPA assay based on enhanced chemiluminescence principle presented very well in screening test for healthy blood donors showing the relative sensitivity and specificity as 100% compared with syphilis IC assay as 100% and 96.5% respectively. VITROS® syphilis TPA assay precision was also excellent at all the three concentrations. Nowadays, several other chemiluminescence based assays are also available, which both IgG and IgM were detected and have been reported 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 [6, 9]. Although our study, VITROS® syphilis TPA showed the both sensitivity and specificity as 100% but the sample size was smaller than other studies in comparison. 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 [10]. These were limitation of our study.

This study also showed that cross-reactivity and interference was not observed and lot-to-lot consistency was studied among all the three lots. The results in table 5 presented that VITROS® syphilis TPA assay has an excellent dilutional sensitivity which was at least 8 times higher than syphilis IC assay. Serologic tests for syphilis is missed by early stage of disease because 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 [6]. The advantage of the chemiluminescence assay is 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. On the other hand, the major advantage of this assay is that it is on an automated platform. In our department with high work-load may benefit from use of an automated screening test on a platform and together with other tests such as anti-HIV, anti-HCV and HBsAg. The number of tests performed on Automated treponemal based immunoassay may be an efficient screening test. An automated assay also decreases the amount of technical time required [7, 8]. 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 [8]. The automated platform and consolidation with other immunoassay testing (HBsAg, anti-HIV and anti-HCV) improves operational efficiency providing rapid results allowing workflows and optimal blood release with an easy documentation and traceability. Vitros 3600 Integrated System 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 3600 Integrated 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.

5. Conclusion

The results suggested that VITROS(®) syphilis TPA should be done to further reduce the risk of transfusion-transmitted TP infection, decrease unnecessary blood waste and loss of blood donors.

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