

COMPARISON OF CHEMILUMINESCENCE IMMUNOASSAY AND IMMUNOCHROMATOGRAPHIC TEST FOR SYPHILIS SCREENING IN BLOOD DONORS ATTENDING A TERTIARY CARE HOSPITAL OF LAHORE

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ABSTRACT

Background and Objective: The screening process of blood donors for transfusion transmitted infections (TTIs) is an essential step of blood transfusion. Multiple tests are available for detection of organism causing syphilis i.e. *Treponema pallidum*. The objective of this study was to compare the performance of Chemiluminescence Immunoassay (CLIA) and Immunochromatographic test (ICT) that are available for this purpose.

Methods: One hundred consecutive samples were obtained from donors presenting in the Blood Bank of Shalamar Teaching Hospital (STH). The screening for syphilis was done using CLIA and ICT simultaneously. The CLIA was performed on VITROS ECi Immunodiagnosics system (Ortho Clinical Diagnostics) and ICT by using Alere Determine Syphilis TP (Abbott Diagnostics, Chicago IL, USA). Sera with discordant results were tested by Fluorescent Treponemal Antibody-Absorption Test (FTA-ABS) for confirmation.

Results: Out of 100 sera tested, 97 were negative and 2 were positive by both the techniques. One sample had discordant result which was reconfirmed using a third method.

Conclusion: Our results showed comparable performance of the two techniques with 99% agreement of results for syphilis screening of the donors. We suggest using the ICT assay in situations (a) where the laboratory cannot afford to have a more advanced system for blood donors, (b) as a backup and (c) in life-threatening situations where time saving may be life saving.

Keywords: Blood Donors, Syphilis Screening, *Treponema pallidum*, FTA-ABS Test, Fluorescent Treponemal Antibody-Absorption Test, Vitros Eci.

INTRODUCTION

Screening for transfusion transmitted infections (TTIs) is a critical step of blood transfusion process as it ensures that transfusion is as safe as possible. To prevent transmission of syphilis via blood transfusion, the World Health Organization (WHO) and International Union against Sexually Transmitted Infections (IUSTI) recommend mandatory screening of all blood donations for syphilis.^{1,2} *Treponema pallidum*, the causative agent of syphilis, is a fragile organism and is sensitive to cold. The risk of transmission through transfusion of blood stored at 2-6°C for more than 72 hours is very low. As the platelet concentrates are stored at room temperature (22°C) or transfused within a few hours of collection, the risk of syphilis transmission is higher with platelet transfusion.³

The serological tests for syphilis are of two types. The Non-treponemal tests e.g. Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) which do not detect antibodies to *T.pallidum*.

These tests detect antibodies to reagin which is a combination of cardiolipin, lecithin and cholesterol. The Reagin is not specific for *T.pallidum* but is generated in response to spirochaete induced damage to cellular membranes and is a useful indicator of the disease activity. *T. pallidum* specific tests e.g. Fluorescent Treponemal Antibody – Absorption Test (FTA – ABS), *T.pallidum* haemagglutination assay (TPHA), enzyme immunoassay (EIA), and CLIA detect antibodies against *T.pal-lidum* antigens.⁴ The ICT uses *T.pallidum* specific antigens to detect antibodies against them in a card or strip format with visual read out.⁵

Previously, non-Treponemal tests were used to screen serum samples for syphilis and positive samples were confirmed by a treponemal assay.⁶ Recently, the WHO, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released an updated algorithm which suggests that in areas having a low disease prevalence; samples may be screened using a treponemal specific

assay and positive samples being analyzed with a non treponemal test to assess disease and treatment status.⁷⁻⁹

This study was designed to evaluate the efficacy of a low cost, easy to perform test instead of a costly and technologically more demanding test so that the screening of blood donors can be performed reliably in urgent situations. The results of this pilot study are reported here and plan to continue the evaluation and comparison of the two tests.

METHODS

This study was carried out prospectively in the Department of Pathology, STH in the month of November 2014.

The consent of the donors was taken before taking their samples to screen for transfusion transmitted infections.

One hundred consecutive samples were obtained from blood donors presenting in the blood bank of STH. These samples were tested by CLIA and ICT simultaneously. Sera with discordant results were tested by FTA – ABS for confirmation.

The CLIA was performed on VITROSE Ci Immunodiagnosics System (Ortho Clinical Diagnostics) in accordance with the manufacturer’s recommendations. VITROS Syphilis TPA assay uses recombinant TP antigen for detection of specific IgM and IgG antibodies. Sera were classified as reactive if the chemiluminescent signal to cut off (S/CO) ratio was ≥ 1.2 .

The ICT was performed by using Alere Determine Syphilis TP (Abbott Diagnostics, Chicago IL USA) with 50 μ l of undiluted serum applied to the sample pad and results were read within 15 minutes. The test was considered positive if red line appeared in the patient window.

RESULTS

Out of 100 sera tested, 97 were negative and 2 were positive by both the techniques (Table 1). Only one sample showed discordant results in the form of a weak positive band present on the ICT strip (compare it with a negative and positive specimens) (Figure 1)

Table 1: Results of Syphilis screening of blood donors (n = 100).

		CLIA*		Total
		Positive	Negative	
ICT*	Positive	2	1	3
	Negative	0	97	97
Total		2	98	100

*CLIA: Chemiluminescence Immunoassay

*ICT: Immunochromatographic test

but non-reactive by CLIA. This sample was further tested by FTA – ABS which turned out to be negative.



Figure 1: Weak positive band present on ICT Strip.

DISCUSSION

Blood transfusion while being lifesaving is not free of risks including the risk of transmission of infection(s) from an infected donor to the recipient¹⁰. Screening for TTIs is an important step of blood transfusion process to ensure the safety of transfusion. The selection of screening tests depends upon a number of factors. Among them, test performance which is measured as sensitivity and specificity is the most important factor while finances, equipment, staff and their expertise, consumables and disposables are also considered during the process of selection.¹¹

The present study was conducted to compare the performance of two treponemal tests *i.e.* CLIA and ICT for screening of syphilis in blood donors. Our results showed comparable performance of the two techniques with 99% agreement of results. After resolution of discordant result, the relative sensitivity and specificity of CLIA was found to be 100%. This is in agreement with the findings of Tiwari *et al*,¹² who have reported 100% sensitivity and specificity of VITROS chemiluminescence assay. Other chemiluminescence based assays like LIAISON assay by Diasorin and ARCHITECT by Abbot are also available. Knight *et al*,¹³ tested 2,645 samples using LIAISON CLIA and reported overall relative sensitivity and specificity as 95.8% and 99.1%. Young *et al*,¹⁴ have reported sensitivity and specificity of ARCHITECT syphilis assay as 98.4% and 99.1% respectively. In another study, Wellinghausen *et al*,¹⁵ evaluated LIAISON and ARCHITECT immunoassays and found their sensitivity as 100% and specificity to be 100% and 99.8% respectively. Chemiluminescence based assays are usually used for screening of blood donors in high volume blood banks due to automation facilities, higher testing throughput and objective interpretation of results, however expensive instrumentation is required for them thus limiting their use in resource limited settings.¹⁶

The ICT performed well in our study. Only one false positive test was observed. Herring *et al*,¹⁷ evaluated nine rapid syphilis ICT kits and reported their sensitivity ranging from 84.5 to 97.7% and specificity

from 93 – 98% when compared against TPHA or *T. pallidum* particle agglutination (TPPA) as reference method/gold standard. A meta-analysis has shown the sensitivity of different ICTs ranging from 85-100% and specificity in the range of 98 – 100%.⁵ ICT is suitable for use in remote and developing regions as they are simple to use, can be transported, stored and performed at room temperature and do not require microscopic or electrical equipment. Moreover they are cheaper and quicker as compared to other treponemal tests.¹⁷

The current study was carried out as a pilot project to compare two treponemal tests for syphilis screening in blood donors. Although the number of samples tested was limited yet we could infer that the two methods had performed equally well and in limited resource settings, the ICT could be used as an alternative for Syphilis screening.

It is **concluded** that our findings demonstrate comparable performance of CLIA and ICT assay for syphilis screening. We suggest using the ICT assay in situations (a) where the laboratory cannot afford to have a more advanced system for blood donors, (b) as a backup and (c) in life – threatening situations where time saving may be life saving.

Authors' Contribution

A. S.: data collection, literature search, material and methods and results writing, contributed to discussion writing. M. R. T.: conceived the idea, literature search, contributed to discussion writing. T. N.: statistical analysis, literature search, contributed to discussion writing. N. A.: contributed to discussion writing. A. M.: data collection, literature search. M.Q.: final review before submission.

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