# Evaluation of an enzyme immunoassay and TPPA for detection of antibodies against Treponema pallidum

*Maharian A[a], Karki S[b], Shrestha S[a] and Raikarnikar M[a]*

## Abstract

**Introduction** An Enzyme Linked Immunosorbent Assay (ELISA) technique is used to screen the Treponemal antibodies in donated blood by many blood banks of all around the world, where as their sensitivity and specificity has been reported to be variable in different population. ELISA techniques have been reported to give less equivocal results and can easily be automatized, making them suitable to be used in Blood Banks, where large number of blood samples need to be screened.

**Objectives** To assess the suitability of an ELISA (Enzygnost Syphilis) test to screen the specific Treponemal antibodies in the units of blood collected from Nepalese Blood donors.

**Methods** This research was conducted in Central Blood Transfusion Service, Nepal Red Cross Society (Exhibition Road). A total of 760 blood samples were randomly collected from blood donors and tested for the presence of specific Treponemal antibodies using ELISA (Enzygnost Syphilis) in an automated ELISA Processor (BEP-III) and TPPA (Serodia TP-PA), strictly following the protocol described in the kit inserts.

**Results** The study evaluated an Enzyme Immunoassay (Enzygnost Syphilis) test for qualitative detection of Specific Treponemal antibodies in 760 sera of blood donors. There was a strong association of test results between the Enzygnost Syphilis and Serodia TP-PA (P-Value <0.000, Fisher's Exact Test). For sera of 9 blood donors the Enzygnost Syphilis was reactive (Sensitivity, 100% in relation to TP-PA) and for sera of 751 blood donors the Enzygnost Syphilis was non-reactive (Specificity, 100% in relation to TP-PA), the overall agreement of the test results was 100 percent (760 of 760 sera ).

**Conclusion** The result suggests that the ELISA test evaluated in this study could be used to screen specific Treponemal antibodies in blood units collected from blood donors.

**Keywords** Screening Treponemal antibodies, Treponema pallidum, ELISA, TP-PA

## Introduction

Treponema pallidum is the dominant pathogen among the spirochetes that causes the venereal syphilis. The incubation period varies from 3-90 days, with a mean of three weeks. The disease encompasses through the stages of primary syphilis, secondary syphilis, latent syphilis and late syphilis[1].

Syphilis can be transmitted by only a few routes: sexual contact[2], direct introduction into vascular system by shared needles or transfusions[3], direct cutaneous contact with infectious lesions, or transplacental transfer of spirochetes. It has been estimated epidemiologically that as many as 50 percent of sexual contacts of infectious persons escape infection[1].

Transfusion transmitted syphilis is not a major hazard of modern blood transfusion therapy. Treponema pallidum, the infectious agent causing syphilis survives at the most for 5 days in blood stored at 4°C[4]. The transmission of syphilis in itself is not a big problem because cure is available for it. However, the presence of a sexually transmitted disease

**Corresponding Author:** Anil Maharjan, MPH, **E-mail:** anilaabhas@hotmail.com, [a]Central blood transfusion service, Nepal Red Cross Society, Exhibition Road, Kathmandu, Nepal. [b]Central Department of Microbiology, TU Kirtipur.

**Table 1: Characteristics of serologic tests for syphilis.**

| | | % POSITIVE AT INFECTIOUS STAGES | | |
|---|---|---|---|---|
| TEST | TYPE | Primary | Secondary | Late |
| VDRL | Nontreponemal | 70 | 99 | 1 |
| RPR | Nontreponemal | 80 | 99 | 0 |
| FTA-ABS | Treponemal | 85 | 100 | 98 |
| TPHA | Treponemal | 65 | 100 | 95 |
| TPI | Treponemal | 50 | 97 | 95 |

Source: Tramont EC, et al. 1990

indicates toward donor's indulgence in "high risk" behavior and consequent higher risk of exposure to infections like HIV and hepatitis which have no cure.

The serologic tests for syphilis can be divided into two groups: non-treponemal tests and treponemal tests. The non-treponemal tests take advantage of antibodies to a tissue lipid, called cardiolipin that are produced as a byproduct of treponemal infection. The procedures in current use are flocculation tests. They use a form of cardiolipin that is complexed with a cholesterol and lecithin. The most commonly used procedures are Veneral Disease Research Laboratory (VDRL) and the rapid plasma regain (RPR) tests. The RPR card tests have achieved popularity because the VDRL test is technically demanding and unforgiving. Other tests that have been used are the reagin screen tests (RST), the unheated serum reagin (USR) test and the toluidine red unheated serum test (TRUST). The non-treponemal tests have a sensitivity of 70-99 percent, depending on the stage of disease[1].

The treponemal tests use specific treponemal antigens into the system. The traditional gold standard was the Treponema pallidum immobilization (TPI) test. This expensive and cumbersome test has been replaced by FTA-ABS test. As this test also requires fluorescent microscope, other easier approaches have been developed. These include Enzyme immunoassays and Particle agglutination test detecting specific treponemal antibodies[5,6,7,8,9,10,11].

## Materials and Methods

A total of 760 blood samples were collected in July to August 2004 from blood donors. All the samples were tested individually for screening of syphilis by using two commercially available screening kits based on different principles viz. Enzygnost Syphilis (Behring, Marburg, Germany) and Serodia TP-PA (Fujirebio, Japan).

The Enzygnost Syphilis is a competitive enzyme immunoassay for the qualitative detection of specific antibodies to Treponema pallidum in human serum or human plasma.

Serodia-TP·PA is a qualitative gelatin particle agglutination assay intended to be used for the detection of Treponema pallidum antibodies in human serum or plasma as an aid in the diagnosis of syphilis.

The principle of Enzygnost Syphilis test is a competitive one stage enzyme immunoassay for the in vitro determination of antibodies to Treponema pallidum. Treponema pallidum specific antibodies (IgG and/or IgM) contained in the sample and the POD labeled antibodies (anti-T. pallidum/POD conjugate) compete for binding to the Treponema pallidum antigens coated into the wells of the microtitration plate. Unbound serum antibodies and conjugate antibodies are washed out and the enzyme activity of the bound conjugate is then determined. The enzyme component of the conjugate reacts with the working chromogen solution (TMB plus hydrogen peroxide), thereby producing a blue colour. The reaction is terminated by the addition of stopping solution POD, resulting in a colour change to yellow. The intensity of the resultant yellow colour is inversely proportional to the concentration of the Treponema pallidum antibodies in the sample. This test was performed according to the recommendation of the manufacturer using the Behring ELISA processor (BEP III) that included incubation, washing, dispensing and reading the result except specimen loading.

The principle of Serodia TP-PA test is based on the agglutination of colored gelatin particle carriers sensitized with T. pallidum (Nichols Strain) antigen. Serum or plasma samples are serially diluted in sample diluent in microplate wells. Sensitized Gelatin Particles are added to respective wells and the contents of the plate mixed by hand or on a tray mixer. The mixture is incubated stationary for 2 hours at room

**Table 2: Qualitative results obtained by Enzygnost Syphilis (ELISA) and Serodia TP-PA.**

| | | Serodia TP-PA | | |
|---|---|---|---|---|
| | | Reactive | Non-reactive | Total |
| Enzygnost Syphilis | Reactive | 9 | 0 | 9 |
| | Non-reactive | 0 | 751 | 751 |
| Total | | 9 | 751 | 760 |

temperature. Serum or plasma containing specific antibodies reacts with the antigen-sensitized colored gelatin particles to form a smooth mat of agglutinated particles in the microtitration tray. A compact button formed by the settling of the non-agglutinated particles characterizes negative reactions. The test is designed to be used exclusively with microtitration techniques. The agglutination patterns and interpretation of the test are clear cut and easy to read visually or with the aid of a tray viewer.

The data of the study was analyzed by Fisher's Exact Test using the statistical software "WinPepi" version 3.8.

## Results

Among the 760 samples tested, 9(1.2%) were reactive both by Serodia TPPA and Enzygnost Syphilis and 751(98.8%) were non-reactive by both the techniques (table 1 and 2). There was a good association of the test result between these two tests during screening of the specific treponemal antibodies in blood donors (P-Value<0.000, Fisher's Exact Test). The samples were also used for evaluation of the sensitivity and specificity of the Enzygnost syphilis (ELISA technique) in relation to the result of TP-PA regarding the TP-PA as "Gold standard." The sensitivity and specificity of the Enzygnost Syphilis (ELISA) was 100 percent compared with the result of Serodia TP-PA. Sensitivity (100%), Specificity (100%), Negative Predictive Value (100%), Positive predictive value (100 %) compared to Serodia TP-PA.

## Discussion

The possibility of using an ELISA technique as an alternative to other formats of treponemal tests has been evaluated in various studies. But there was no reliable data that support the use of an ELISA technique, as a screening test to detect the specific treponemal antibodies among Nepalese blood donor population. So, in this study, only the sera of blood donors, undergoing stringent donor screening and selection criteria was used. The Serodia TP-PA was used as Bench-mark to compare the ELISA (Enzygnost Syphilis) and to evaluate the relative sensitivity and specificity because of a number of reasons like, the Serodia TP-PA has been described to be produced using the same antigens, the high performance liquid chromatography purified sonicate of Treponema pallidum, that are used in a reliable and established confirmatory test for Syphilis, the MHA-TP(Serodia TP-PA, Kit insert). The Serodia TP-PA has been shown to be more sensitive and specific than many ELISA techniques in a number of studies particularly during the secondary and latent stage of the disease [12]. The Serodia TP-PA has also been used by Center for Disease Control and prevention (CDC) as a confirmatory test to diagnose Syphilis[13]. In this study, the ELISA (Enzygost Syphilis) showed a sensitivity and specificity of 100 percent ,which is slightly higher than in the study published by Virivatavikul R. et al[14].(i.e. sensitivity of 99.1 and specificity of 98.91 percent when the equivocal value was considered negative and sensitivity of 100 percent and specificity of 97.89 when the equivocal value was considered positive) which may be due to testing of higher number of samples in that study (i.e. 2882), some of which were reported to be previously known positive samples by VDRL, TPHA or FTA-ABS tests, probably representing the more diverse population and stage of disease. Similarly, slightly less sensitivity of Enzygnost Syphilis was reported by Maidment C. et al. (i.e. 99.5%) compared with the result of TPHA[16]: this was probably due to the use of sera which were recognized as causing problems with enzyme immunoassays. But the test specificity observed in our study was totally in concordance with the study conducted by Microbiological Diagnostics Assessment Service (MiDAS)[15] and as claimed by the Dade Behring Company (kit insert) while testing the samples of Blood donors .Thus, on the basis of present study and similar studies[11,15,16], ELISA (Enzygnost Syphilis) could be used as a screening test to detect specific treponemal antibodies in sera from healthy blood donors, where large number of donated blood need to be tested.

## Conclusion

The current study has demonstrated that the ELISA (Enzygnost syphilis) is highly sensitive and specific

screening method to screen specific treponemal antibodies in donated units of blood, while compairing it with the test results by Serodia TP-PA. Since, the ELISA tests can easily be automated, human errors would be minimized and test reports can be timely released, improving the quality of service where large numbers of blood units have to be screened. Moreover, ELISA technique is relatively cheaper than TP-PA for screening specific traponemal antibodies.

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