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

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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 (Enzymes 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 (Enzymes Syphilis) in an automated ELISA Processor (BEPIII) and TPPA (Serodia TP-PA). strictly following the protocol described in the kit inserts.

Results

The study evaluated an Enzyme Immunoassay (Enzymes 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 Enzymes Syphilis and Serodia TP-PA (P-Value <0.000, Fisher’s Exact Test). For sera of 9 blood donors the Enzymes Syphilis was reactive (Sensitivity, 100% in relation to TP-PA) and for sera of 751 blood donors the Enzymes 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 syphilis1.

Syphilis can be transmitted by only a few routes: sexual contact2, direct introduction into vascular system by shared needles or transfusions3, 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 infection1.

Transmission 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°C4. 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
Table 1: Characteristics of serologic tests for syphilis.

<table>
<thead>
<tr>
<th>TEST</th>
<th>TYPE</th>
<th>% POSITIVE AT INFECTIOUS STAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL</td>
<td>Nontreponemal</td>
<td>70</td>
</tr>
<tr>
<td>RPR</td>
<td>Nontreponemal</td>
<td>80</td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>Treponemal</td>
<td>85</td>
</tr>
<tr>
<td>TPHA</td>
<td>Treponemal</td>
<td>65</td>
</tr>
<tr>
<td>TPI</td>
<td>Treponemal</td>
<td>50</td>
</tr>
</tbody>
</table>

Source: Tramont EC, et al. 1990

The Enzyvenost 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 Enzyvenost 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 microtiter 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 includes incubation, washing, dispense 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 microwell plates. 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

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. Enzyvenost Syphilis (Behring, Marburg, Germany) and Serodia TP-PA (Fujiirebio, Japan).
temperature. Serum or plasma containing specific antibodies react 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 trav 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 Enzvenost 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 Enzvenost syphilis (ELISA technique) in relation to the result of TPPA regarding the TP-PA as “Gold standard.” The sensitivity and specificity of the Enzvenost 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 Benchmark to compare the ELISA (Enzvenost 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 Trenonema 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 confirmatory test to diagnose Syphilis13. In this study, the ELISA (Enzvenost Syphilis) showed a sensitivity and specificity of 100 percent which is slightly higher than in the study published by Virvatavikul R, et al14, (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 Enzvenost Syphilis was reported by Maidment C, et al (i.e. 99.5%) compared with the result of TPHA14; 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 studies11,15,16, ELISA (Enzvenost 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 (Enzvenost syphilis) is highly sensitive and specific.
screening method to screen specific treponemal antibodies in donated units of blood, while concomitantly 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 treponemal antibodies.

**Acknowledgement**

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**References**


