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Syphilis Diagnosis Using an Advance Concept for Non-Treponemal Test Development

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Abstract

Today, different laboratory techniques are available for syphilis diagnosis. Among all, serological tests are the most popular because of their ease of use. Serological tests for syphilis diagnosis can be grouped into non-treponemal and treponemal types. The non-treponemal test type includes Venereal Disease Research Laboratory (VDRL) slide test, Unheated Serum Reagin (USR) test, Rapid Plasma Reagin (RPR) test and Toluidine Red Unheated Serum test (TRUST). All detects non-treponemal antibody and were introduced many years back. Still they are use for the syphilis screening in India. Treponemal test detects treponemal specific antibody and are available in different assay formats. Recently, introduction of automated treponemal test had changed the practice for the syphilis diagnosis. Many laboratories are now following reverse practice; automated treponemal test is use first for syphilis screening. Non-treponemal tests are not available in automated format. However, their availability can be very useful in syphilis diagnosis. It is ideal for the huge number of sample study especially in blood bank settings where work load is more. In current study, an attempt was made to develop a non-treponemal test which can be automated. Cardiolipin antigen was modified and coated on microwell surface through an advance concept of covalent attachment. Such antigen coated plate was studied with few syphilis reactive samples in enzyme immunoassay (EIA) format. It was found out that cardiolipin got attached to microwell surface successfully and showed promising result with end-point titer determination study with syphilis reactive samples. RPR was used as reference test. Both tests demonstrated similar result in their ability to determine the end-point titer. Further study is required with more number of samples to validate test performance for syphilis diagnosis. Described test can be useful as substitute for currently used non-treponemal tests.

Keywords: Syphilis,VDRL,USR,RPR,TRUST, EIA

Syphilis is a disease caused by a spirochete bacterium *Treponema pallidum* subspecies *pallidum.* Major route of transmission is through sexual contact. However, it may also transmitted by other modes which includes vertically from mother to fetus during pregnancy or at birth resulting into congenital syphilis, through blood transfusion and non-sexual contact (1). Therefore, syphilis infection can be either acquired or congenital type. Syphilis presents different clinical signs and symptoms. It can be classified as primary, secondary, latent, and late or tertiary (2).

The causative agent of syphilis cannot be grown on artificial culture media (3). This makes difficult in bacterial identification and characterization through routine microbiological culture techniques. However, many methods are available for syphilis diagnosis which includes

dark field microscopy, serological tests and direct antigen detection tests (4). Among all, serology is the most frequently used method to diagnose syphilis (3, 5). Non-treponemal serological tests like VDRL, USR, RPR and TRUST have been used for syphilis diagnosis since long. Different treponemal specific serological testes are also available.

It includes *T.pallidum* Particle Agglutination Assay (TP-PA), *T.pallidum* Hemagglutionation Assay (TPHA), Fluorescent Treponemal Antibody Absorption Assay (FTA-ABS) and Enzyme Immunoassay (EIA) that detects IgG or IgM or both classes of antibody to treponemal proteins. Treponemal tests are available in automated format which is an added advantage over the non-treponemal test. Due to this, many laboratories had changed their practice to use treponemal test first for sample screening (6). Both non-treponemal and treponemal tests are useful for syphilis diagnosis. Though, both have their own limitations for syphilis diagnosis.

Antigen preparation of non-treponemal tests uses natural or synthetic cardiolipin along with lecithin and cholesterol. This antigen preparation reacts with non-treponemal antibodies from syphilitic patients and shows flocculation. Advantages of this type of test include easy to use, economical, rapid resultand easy to monitor efficacy of treatment (4). However, there are some conditions like autoimmune diseases, pregnancy, viral infections which may give false positive test result (4). Therefore, a reactive test result always need to be confirmed with a treponemal test as per traditional algorithm for syphilis diagnosis (7). Still all non-treponemal tests are being performed manually as none of them are available in automated format which serves as their limitation over the automated treponemal assays.

Many researchers tried to develop non-treponemal EIA system due to its potential for automation. Such type of system is suitable for large scale sample study. An EIA system capable of detecting IgG and IgM classes of antibody to VDRL antigen was reported in earlier study which had claimed its sensitivity and specificity equivalent to traditional non-treponemal tests (8). A urease enzyme based assay system for the detection of IgG class of antibody to VDRL antigen was also reported. This system demonstrated sensitivity and specificity of 96.6% and 99.6% respectively (9). Both EIA systems were developed with VDRL antigen which was dried on microwell surface and subsequently used in assay development. A different concept was reported in which cardiolipin antigen was modified and attached to wide varieties of molecules like KLH, BSA, IgY, synthetic proteins, biotin, streptavidin or avidin (10). This is a modern concept for cardiolipin modification and its attachment to different bio-molecules or to the solid support directly. Cardiolipin-protein or cardiolipin attached directly to microwell can use for non-treponemal antibody detection. This method fixes antigen on microwell surface very strongly through covalent linkage and can give consistent test result.

Oxidized cardiolipin preparation was obtained from Dr. Castro (CDC) for evaluation and study (10). This antigen was attached directly to microwell plate with primary amine group on it. Such type of plate can be purchased commercially from different suppliers like Biomat (Italy) and Becton Dickinson (USA). Cardiolipin was attached to microwell by following similar method that was used originally for conjugating it with protein (10). Cardiolipin coated plate was tested later (8). Tetramethylbenzidine was used as chromogen in testing protocol due to its better sensitivity over o-phenylenediamine (11). Reaction was stopped with sulfuric acid (8) and read at 450 nm wavelength. A strong reactive sample (RPR titer of 128) and a sample with moderate reactivity (RPR titer of 8) were used for testing. Each syphilis reactive sample was diluted serially in normal human serum to two fold and tested. A syphilis non-reactive sample was also run as a negative control in this study. Obtained response of samples with non-treponemal EIA was shown in figure 1.

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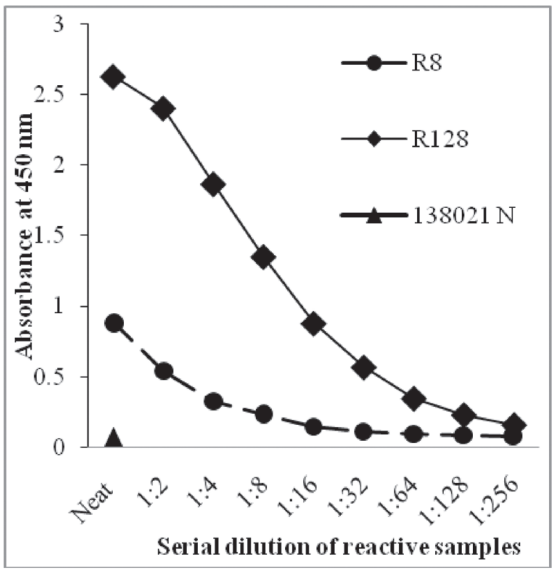

Fig. 1 End-point titer determination study with two syphilis reactive samples in developed non-treponemal EIA test**.**

Response of tested syphilis reactive samples with cardiolipin coated microwell demonstrated that cardiolipin got attached to microwell successfully. Decrease in absorbance was observed when syphilis reactive sample was diluted serially and tested (as shown in Fig. 1). Syphilis reactive samples were tested in RPR test kit before (ASI, USA) to determine their end-point titer. Testing was done as per manufacturer's instructions for semi-quantitative assay. The Result of non-treponemal EIA and RPR test were found out to be similar in their ability to determine end-point titer of studied reactive samples. Non-reactive sample (138021N) remained non-reactive with both tests.

In summary, sero-diagnosis of syphilis involves testing with non-treponemal and treponemal tests. Selection of a screening test depends upon acceptance of practice for syphilis diagnosis. Traditional practice uses non-treponemal test first for syphilis screening and a treponemal test later as confirmatory test. In contrast to that, reverse practice uses treponemal test first for screening and a non-treponemal test later for confirmation.Though, reverse practice was reported to yield more false positive test result than the traditional practice when it was studied with low-prevalence syphilis population (12). Treponemal antibody remains present even after successful treatment and which may give reactive test result with treponemal specific tests (5). Despite that, high sensitivity and specificity of treponemal tests for syphilis diagnosis was reported (3).

Described study demonstrated promising result with cardiolipin coated microwell prepared though an advance concept of covalent coupling. Antigen was attached to the plate surface via its tail. This may expose the head group of antigen molecule so that non-treponemal antibody can recognize it well. This enzyme immunoassay has potential for automation and may be very useful in order to satisfy the long-term need for the automated non-treponemal test. Further study is required with more number of samples with and without syphilis in order to validate the performance of this concept.

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