Comparison Of Three Methods Of Syphilis Testing In Blood Donors-RPR, TPHA & ELISA

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Abstract: Background: syphilis can be transmitted through blood, so it is significant to choose a method with high specificity and affinity to test syphilis when do the blood screening. The goal of this study was to compare the performance of three commercially available treponema-specific assays using 58,969 serum samples collected in our blood bank. Material And Methods: Serum samples collected in our blood bank were tested with the 3 treponema-specific assays described below. each sample was tested with an RPR, TPHA and ELISA to assess potential recent infection. 58,969 serum samples were collected over the period of 1.5 year and tested for syphilis in our blood bank. blood units showing reactivity in any one of the tests, were discarded as per NACO strategy I for blood bank. Result: The total samples of three methods are 58,969.Among the total samples studied, 125(0.22%) were reactive by the RPR technique, while 331 (0.56%) samples, were reactive by the ELISA & TPHA techniques, So there were 125 samples which were reactive by all 3 methods. However, the 206 samples which are negative through RPR and are tested positive in ELISA and in TPHA. Conclusion: data suggest that each method has limitations. It is important that health care providers must perform a thorough review of each patient's clinical and treatment history when interpreting the results of syphilis serology. There are statistical differences on positive rate when the 206 blood samples are tested through syphilis RPR, TPHA and syphilis ELISA. Compared with the results of TPHA and ELISA, RPR have both false positivity and false negativity but the results of TPHA and ELISA just have false positivity. In addition, detection capability of syphilis ELISA and of TPHA is stronger than RPR syphilis and it also be more fitting to blood screening. So in comparison of methodology and practical operation RPR are not fit for the large-scale blood screening while syphilis ELISA and TPHA is the one which can be applied to a large-scale blood screening. [Shah M Natl J Integr Res Med, 2020; 11(3):15-18]

Key Words: ELISA, RPR test, Syphilis, TPHA, Treponema pallidum

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Introduction: The diagnosis of syphilis is often based on the results of serology using assays designed to detect either nontreponemal (e.g., rapid plasma reagin [RPR] and VDRL) or treponema-specific antibodies (e.g., EIAs, TPHA, fluorescent treponemal antibody [FTA-abs] test).
It is known that syphilis can be transmitted through blood, so it is significant to choose a method with high specificity and affinity to test syphilis when do the blood screening. This study aims to do a comparison among RPR,TPHA, ELISA. The following are the analysis of the three methods to test syphilis in blood sample and the conclusion of the most fitting method to have a large-scale blood screening. For the detection of syphilis the following three methods work well. ELISA is used for automatic detection, RPR and TPHA for manual testing.

Material & Methods: In our study, we have taken donor samples for testing. Before donation, we have taken signature of each donor on consent form which along with permission of voluntary blood donation also mentions their permission to perform the tests for study purpose. Serum samples collected in our blood bank were tested with the 3 treponema-specific assays described below. Each sample was tested with an RPR, TPHA and ELISA to assess potential recent infection. 58,969 serum samples were collected over the period of 1.5 year and tested for syphilis in our blood bank. Samples were stored at 4°C until all testing was complete so that analyses were performed in the same freeze-thaw cycle. Blood units showing reactivity in any one of the tests, were discarded as per NACO strategy I for blood bank.

Enzyme Linked ImmunoSorbentAssay: All serum samples were tested according to the manufacturer's instructions using EIAs for detection of total antibodies(IgG and/or IgM) against trepenoma pallidum(Tp) in human serum or plasma and utilizes the recombinant treponemal antigens Tp47, Tp17, Tp15, and Tp44 (TmpA).(Erba Lisa syphilis) some tests of ELISA are being performed using kits of bio-rad which detects antibody to treponema pallidum in

human serum or plasma. The results are calculated as index values (optical density of sample/cut off value) and are then classified as negative (<1.0) or positive (≥1.0). All testing by EIA was performed on automated analyzer and reader (Evolis). Enzyme immunoassays have shown some advantages in relation to the tests used for the laboratory diagnosis of syphilis [1, 2, 3, 4], since they are easy and quick to perform and objective to read. They also have the potential to be automated.

Rapid Plasma Reagin Assay: Testing by the RPR assay was performed according to the manufacturer's instructions using modified slide test for syphilis (Beacon diagnostics pvt. Ltd.). Serum samples were tested undiluted, and in addition, a 2-fold dilution series was prepared using 0.9% sodium chloride diluent as outlined in the manufacturer's instructions.

Treponema Pallidum HemAgglutination: Samples were tested with the TPHA assay according to the manufacturer's instructions (Meril). This assay is based on the agglutination of colored gelatin particles that have been sensitized (coated) with T. pallidum (Nichols strain) antigen. Testing and result interpretation were performed in strict accordance with the recommendations outlined in the manufacturer's instructions.

Results: Among the 58,969 samples studied, 125(0.22%) were reactive by the RPR technique, while 58,844(99.78%) were nonreactive. By the ELISA & TPHA techniques, 331 (0.56%) samples were reactive while, 58,638 (99.43%) samples were nonreactive. So there were 125 samples which were reactive by all 3 methods. The total samples of three methods are 58,969. However, the 206 samples which are negative through RPR and are tested positive in ELISA and in TPHA.

Table 1: Sample Of Three Methods

Results	Test		
	RPR	SYPHILIS	TPHA
Reactive	125	331	331
Non-Reactive	58,844	58,638	58,638

Discussion: The phospholipid antibodies detected by nontreponemal tests are not only produced in syphilis and other treponemal disease but also in response to a variety of conditions unrelated to syphilis. Therefore, false-positive nontreponemal test reactions can have multiple causes. Their incidence is generally 1% to 2%. The rate of false-positives during pregnancy is no greater than that seen in the general population, but is higher among intravenous drug users. Generally, up to 90% of false-positive reactions have a titre of less than 1:8, and reactive nontreponemal tests with titres less than 1:8 and subsequent nonreactive treponemal tests are considered to be biological false-positive reactions. Chronic false-positive reactions persist for more than six months and are often associated with autoimmune disorders and chronic inflammatory conditions. False-positive reactions can also occur with treponemal tests but this is less common than with nontreponemal tests.

Both types of tests can also yield false-negative results due to the prozone phenomenon. Such false-negatives occur in 1% to 2% of patients, especially in pregnant women and HIV patients. Serum from such patients should be tested at a 1:16 dilution. This, however, requires that the laboratory is given the relevant information from the patient's history and clinical diagnosis. When the sensitivities of the ELISA, RPR test, and TPHA were compared, the ELISA and TPHA technique had a higher sensitivity, although the difference was not statistically significant, during all phases of disease, although other investigators have found that the ELISA and TPHA method has a lower sensitivity during the primary stage of syphilis [5].

Since detection of anti-T. pallidum IgM antibodies is important in the differentiation between recent and late infection, we decided to verify using both the ELISA and the TPHA techniques whether IgM antibodies were present in patients with recent stages of syphilis. It seems that the majority of samples with indeterminate reactivities obtained were indeed reactive. This fact demonstrates the utility of this ELISA as a marker of recent infection. The results of this study show that the ELISA may be an alternative to the treponemal tests for the detection of T. pallidum antibodies, including the presence of IgM, since it has sensitivity and a specificity similar to those of the most commonly used tests during all stages of syphilis. This was especially true when the ELISA technique is considered the most sensitive and specific test for the diagnosis of syphilis. The ELISA and TPHA also had

sensitivity similar to that of the RPR test, having the advantage of presenting no false-positive results.

We think that the enzyme immunoassay and TPHA technique studied here could be used as a screening test, also EIA is simple, objective, and easily automated. Recent updates to the syphilis testing algorithm propose the use of a treponema-specific assay (e.g., EIA) for screening purposes, with positive samples being analyzed by a nontreponemal test[6]. This paradigm shift represents a reversal of a long-held practice and has generated substantial confusion among health care providers and patients. Despite our findings showing comparable performance of the 3 treponemal assays, there were samples with discordant results that became a focus for further investigation. In order to potentially resolve these discrepancies, we reviewed the results of all other treponemal tests, as well as those of the RPR and IgM assays, to determine the likelihood of past or recent infection.

This study has several limitations. First, the serum samples were collected without corresponding clinical data, so we were unable to correlate results to the clinical presentation or treatment history. Despite this, each sample was analyzed by 3 treponemal assays, as well as IgM and RPR assays, and this allowed for a robust characterization of the serologic status of each sample. A second limitation of our study is that a subset of the serum samples was selected based on prior results, and therefore, we could not determine the positive and negative predictive values of each test. Third, the results from this study do not address whether screening with a treponema-specific assay is clinically or economically advantageous compared to screening by RPR assay. Past reports have suggested advantages and limitations to both strategies[7,8], and further studies are needed.

Interestingly, among the 58,969 serum samples tested in our study, 125(0.22%) were reactive by the RPR technique, while 58,844(99.78%) were nonreactive. By the ELISA & TPHA techniques, 331 (0.56%) samples were reactive while, 58,638 (99.43%) samples were nonreactive. This has important clinical implications, as treponema-specific assays may be positive in patients with either active syphilis or past, successfully treated disease. Therefore, it is often difficult to determine the significance of reactive treponemal screening results when non-treponemal tests are negative, especially in patients without a history of treatment for syphilis. This can complicate the interpretation of results and may lead to higher rates of treatment compared to screening with a nontreponemal test [9].

Conclusion: Our findings demonstrate comparable performance among the 3 treponema-specific assays evaluated. However, our data suggest that each method has limitations. It is important that health care providers must perform a thorough review of each patient's clinical and treatment history when interpreting the results of syphilis serology. There are statistical differences on positive rate when 206 blood samples are tested through syphilis RPR, TPHA and syphilis ELISA. Compared with the results of TPHA and ELISA, RPR seems less accurate in finding syphilis. In addition, detection capability of syphilis ELISA and of TPHA is stronger than RPR syphilis and it also be more fitting to blood screening. So in comparison of methodology and practical operation RPR is not fit for the large-scale blood screening while syphilis ELISA and TPHA is the one which can be applied to a large-scale blood screening.

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