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Clinical Utility of a Competitive ELISA to Detect Antibodies Against *Treponema pallidum*

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Screening for *Treponema pallidum* infection is carried out on a large human population. To reduce costs, fewer tests which still offer adequate sensitivity and specificity could be performed. We studied the reliability of a novel indirect ELISA method to test for this infection. Several panels of sera were used that corresponded to 40 primary infections (group 1), 13 recurrences (group 2), 348 latent infections (group 3), 5 samples with anticardiolipin antibodies (group 4), 15 samples from patients with Lyme borreliosis (group 5), and 400 samples from blood donors and healthy pregnant women (group 6). The ELISA showed a global sensitivity and specificity of 100 and 99.5%, respectively. Our evaluation shows that Enzygnost Syphilis is a sensitive, specific, and simple test to screen for this infection. J. Clin. Lab. Anal. 14:83–86, 2000. © 2000 Wiley-Liss, Inc.

Key words: *Treponema pallidum*; antibody; ELISA

INTRODUCTION

The diagnosis of *Treponema pallidum* (Tp) infection is usually carried out by serologic methods. These include tests with nontreponemal antigen (rapid plasma reagin, RPR), whole bacteria—either purified (microhemagglutination assay, MHA-TP) or not (fluorescent treponemal antibody-absorption test, FTA-Abs)—and recombinant proteins. Laboratories mostly use the RPR and MHA-TP or FTA-ABS, or only the RPR (1). The drawbacks of the RPR include the false positives and false negatives at the very early and late latent stages of the disease. Neither RPR nor MHA-TP (or FTA-Abs) tests can be automated, and they are sometimes difficult to interpret. Finally, the simultaneous or successive use of multiple tests improves diagnostic accuracy but increases health costs (2–4).

ELISA tests that can be readily automated (BioELISA Syphilis, Captia Syphilis, Enzygnost Syphilis, and ICE Syphilis) have recently become available (5–9). They could reduce the laboratory work but their utility to detect the infection in its different stages must be demonstrated. Our aim was to determine the value of a single ELISA test (Enzygnost Syphilis, DadeBehring, Germany) to detect the maximum number of cases of this infection with adequate sensitivity and specificity, and to propose a protocol for the diagnosis of the infection that uses automated methods and the minimum number of nonautomated complementary tests.

MATERIAL AND METHODS

Testing Protocol

A total of 821 serum were included in the evaluation, divided between several groups. Group 1 consisted of 40 sera from patients with primary infection with Tp, defined by the presence of chancre compatible with primary syphilis, RPR ≥ 16 (Macro-Vue RPR, Becton Dickinson Microbiology, Cockeysville, MD), MHA-TP at a titer ≥ 640 (Cellognost Syphilis, DadeBehring), FTA-Abs (+) (Trepo-Spot IF, Biomerieux, France), and age 35 ± 5 years old. Group 2 was comprised of 13 serologic recurrences, defined by a clinical history of syphilis and ≥ 4-fold increase in RPR titer between two samples and age 37 ± 5 years old. Group 3 was comprised of 348 sera from patients with asymptomatic latent infections, defined by a clinical history of syphilis and who are currently RPR-negative, MHA-TP with positivity at a titer of 80–160, and age 60 ± 10 years old. Of these, 136 were from patients with early latent syphilis (< 1 year of clinical evolution and FTA-Abs positive) and 212 from those with late latent syphilis (> 1 year clinical evolution and FTA-Abs negative). Group 4 was comprised of five sera from individuals without syphilis and with anticardiolipin antibodies (autoimmune diseases, age 45 ± 6 years old). Group 5 was comprised of 15 sera from patients with arthritis due to *Borrelia burgdorferi* and with no clinical history of syphilis (age 30 ± 5 years old). All these patients had a history of a tick-bite, articular disease in knee or elbow, and had IgG anti-*B. burgdorferi* in serum according to CDC interpretation criteria (10). Group 6 was comprised of 400 samples from blood

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donors and healthy pregnant women. Groups 4, 5, and 6 were used as negative control groups.

In all samples anti-Tp antibodies were determined by the ELISA test under study (Enzygnost Syphilis).

Serologic Methods

The RPR, MHA, and the FTA-ABS were performed according to the manufacturers' instructions (5). The ELISA was performed first with MegaFlex automated pipetting and then with the Behring ELISA Processor system (BEP III) (DadeBehring). Briefly, 25 μL of specimens from patients and controls (four negative and two positive controls) were transferred to microtiter wells. Enzygnost Syphilis is a competitive one-stage enzyme immunoassay with *T. pallidum* Nichols strain (inactivated) detergent extract antigen. Tp-specific antibodies (IgG and/or IgM) contained in the sample and the peroxidase-labeled antibodies (POD) (anti-Tp/POD conjugate) compete for binding to the Tp antigens coated onto wells of the microtitration plates. 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 (tetramethylbenzidine (TMB) plus hydrogen peroxide), thereby producing a blue color. This reaction is terminated by the addition of stopping solution POD, resulting in a color change to yellow. The intensity of the resultant yellow color is inversely proportional to the concentration of Tp antibodies in the sample. The manufacturer recommends that samples with equivocal ELISA results be retested. However, no specimens tested in this study had equivocal results.

The *chi*-square test was used to assess the significance of the differences in performance criteria between the various tests.

RESULTS

The results are listed in Table 1. We have obtained 100% positive results by Enzygnost in samples from groups 1 (primary infections), 2 (recurrences), and 3 (latent infections). We have not obtained any positive results (0%) by Enzygnost in samples from groups 4 (autoimmune diseases) and 6 (blood donors and healthy pregnant women). In samples from group 5 (Lyme borreliosis) we have obtained 13.3% positive results by Enzygnost. There were no samples with equivocal results in the present study. There was a good correlation between results obtained by Enzygnost and the status of patients to Tp in panels 1, 2, 3, 4, and 6. In panel 5, antibodies to Tp with Enzygnost were present in two (13.3%) acute infections with *B. burgdorferi*. Enzygnost was present in patients with late latent infection and negative FTA-ABS ($P < 0.01$). There were no cases of prozone phenomenon in our sample. The sensitivity, specificity, and positive and negative predictive values of Enzygnost for the diagnosis of past or recent primary Tp infection in the present series were 100, 99.5, 99.5, and 100%, respectively. This result was obtained when infected subjects were compared to noninfected subjects (Table 1).

TABLE 1. Results of the Enzygnost Syphilis test on the groups in the study

Group	Number in group	Enzygnost (% positive)
1 Primary infections	40	100
2 Recurrences	13	100
3 Latent infections	348	100
4 Samples with anticardiolipin antibodies	5	0
5 Patients with Lyme borreliosis	15	13.3
6 Blood donors and healthy pregnant women	400	0

DISCUSSION

The definitive diagnosis of infection with Tp requires several laboratory tests. Different assays can be used depending on the stage of the infectious process, i.e., primary infection, reactivation, or chronic infection (3,8). Costs could be reduced if a smaller number of tests were used. The detection of anti-Tp antibodies requires the use of specific antigens with the greatest immunogenic power to reduce the number of false positives and negatives. The detection of syphilis in its tertiary stage and epidemiological studies of the infection are compromised when only nontreponemal tests are employed. The FTA-ABS and RPR tests can give false negative results after many years in patients with late latent infection. Finally, automated tests are desirable to save time and improve the reproducibility of results. The present study contributes our nine-year experience using a competitive ELISA test with purified whole antigen (Enzygnost Syphilis) for the diagnosis of infection with Tp at different stages. The high sensitivity and specificity of the EIA in the present study are similar to the findings in two previous studies that used EIA alone to diagnose syphilis, reporting sensitivities of 93.9 and 100% and specificities of 98.6 and 98.2% (6,7). Furthermore, in our series, the ELISA test was more sensitive than the FTA-ABS in the sera of subjects with late latent infection and as sensitive as MHA. Young et al. (9) had similar results using an ELISA test with recombinant antigens. It has been reported that recombinant antigens appear to be more specific than whole antigens, whether the latter are purified or not. Thus, assays with ICE Syphilis (Abbot), with three recombinant Tp antigens (TpN15, TpN17, TpN47) achieved better results than did those with Captia Syphilis (Centacor), which uses the native Tp antigen (9). However, in the present study we only found false positives in patients with Lyme borreliosis.

In previous studies (1,5,7,9), some ELISA tests were assessed but without establishing infection diagnosis guide-

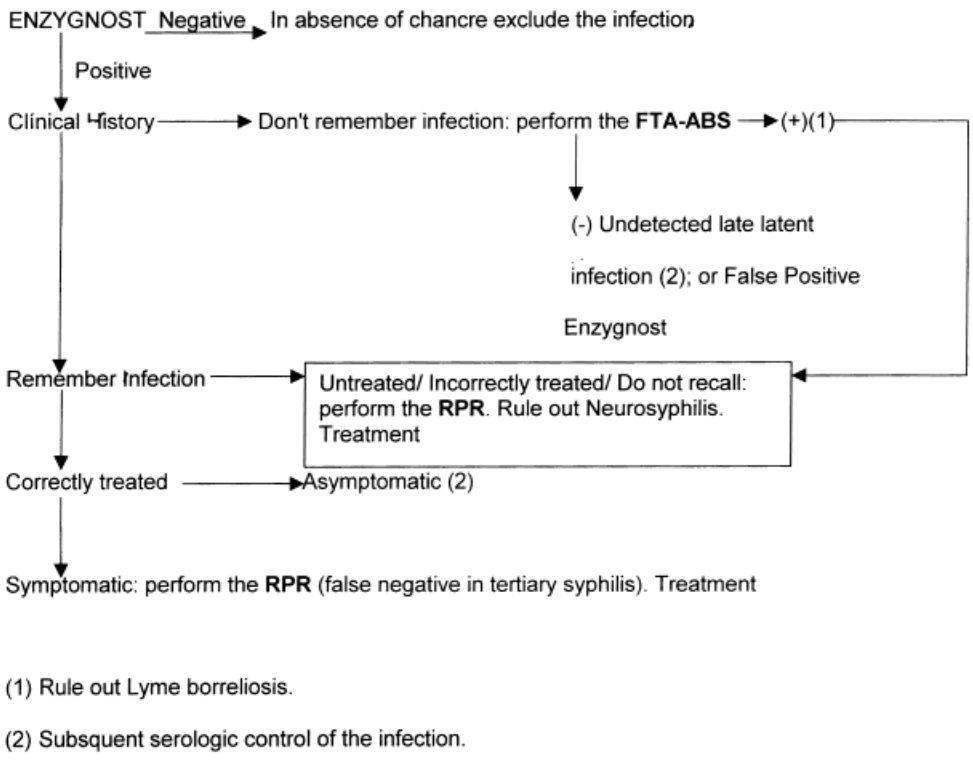

Fig. 1. Diagnosis protocol using Enzygnost Syphilis as the principal test.

lines for their use as a screening test. Following the completion of the present evaluation, we proposed a protocol to be followed for the Enzygnost test (Fig. 1) that may improve its performance and save laboratory costs and time. It implies coordinated action with the clinician, but if direct communication is not possible and a positive result is obtained with Enzygnost, it is adequate (1) to perform the RPR to test for active disease; and (2) to signal the possibility of a false positive by Enzygnost due to infection with other spirochetes. Since equivocal results were not obtained in our study, we cannot establish a protocol for such cases, but the manufacturer recommends repeating the test. If the same result is obtained, a new sample must be requested. The high-specific antibody indexes obtained with the Enzygnost Syphilis test and its high sensitivity indicate its use to detect or exclude treponemal infection in patients infected with HIV. However, the HIV status of the patients in the present study was unknown. The fact that Enzygnost is based on a competitive ELISA offers two additional advantages: (1) a greater specificity; and (2) a single ELISA test can detect IgG and IgM. Another commercial test (BioELISA, Biokit, Spain) is similar to Enzygnost but differs in that it utilizes a human anti-Tp IgG peroxidase-labeled conjugate for specific antigenic sites from the Nichols strain. This may compromise its sensitivity to detect the infection at early stages (5). Nevertheless, the false diagnoses produced in our study may suggest a need for further research on this issue, since infection with *B. burgdorferi* is relatively prevalent in some areas and may lead to a false diagnosis of infection with Tp.

In conclusion, the Enzygnost system is an efficacious method to detect Tp infection, with the caveat that false diagnoses may be produced. Because of this possibility, we propose the following additional tests when a positive result is obtained: (1) a nontreponemal test prior to antibiotic therapy as a baseline to monitor the clinical response; and (2) the FTA-ABS in the few cases where the patient's clinical history casts doubt upon a positive result by the Enzygnost Syphilis test.

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