

Clinical Comparison of the *Treponema pallidum* CAPTIA Syphilis-G Enzyme Immunoassay with the Fluorescent Treponemal Antibody Absorption Immunoglobulin G Assay for Syphilis Testing

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Received 4 March 1999/Returned for modification 4 June 1999/Accepted 29 June 1999

Recently, a treponema-specific immunoglobulin G (IgG) enzyme immunoassay (EIA), the CAPTIA Syphilis-G (Trinity Biotech, Jamestown, N.Y.), has become available as a diagnostic test for syphilis. A total of 89 stored sera previously tested by the fluorescent treponemal antibody absorption (FTA-ABS) IgG assay were evaluated by the CAPTIA EIA. The FTA-ABS IgG procedure was performed by technologists unblinded to results of rapid plasmid reagin (RPR) testing of the same specimens. Borderline CAPTIA-positive samples (antibody indices of ≥ 0.650 and ≤ 0.900) were retested; if the second analysis produced an index of >0.900 , the sample was considered positive. Thirteen of 89 (15%) samples had discrepant results. Compared to the FTA-ABS assay, the CAPTIA EIA had a sensitivity and specificity and positive and negative predictive values of 70.7, 97.9, 96.7, and 79.7%, respectively. In another analysis, discrepancies between results were resolved by repeated FTA-ABS testing (technologists were blinded to previous RPR results) and patient chart reviews. Seven CAPTIA-negative samples which were previously interpreted (unblinded) as minimally reactive by the FTA method were subsequently interpreted (blinded) as nonreactive. One other discrepant sample (CAPTIA negative and FTA-ABS positive [at an intensity of 3+], unblinded) was FTA negative with repeated testing (blinded). For the five remaining discrepant samples, chart reviews indicated that one patient (CAPTIA negative and FTA-ABS positive [minimally reactive], blinded) had possible syphilis. These five samples were also evaluated and found to be negative by another treponema-specific test, the *Treponema pallidum* microhemagglutination assay. Therefore, after repeated testing and chart reviews, 2 of the 89 (2%) samples had discrepant results; the adjusted sensitivity, specificity, and positive and negative predictive values were 96.7, 98.3, 96.7, and 98.3%, respectively. This study demonstrates that the CAPTIA IgG EIA is a reliable method for syphilis testing and that personnel performing tests which require subjective interpretation, like the FTA-ABS test, may be biased by RPR test results.

At our institution, like many others, the laboratory diagnosis of syphilis is achieved by first screening serum with a nontreponemal test and then confirming positive results with a treponema-specific test. The most commonly used nontreponemal tests are the rapid plasmid reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) assays. To confirm positive results obtained with these nontreponemal tests, one of two treponema-specific tests is frequently used: the fluorescent treponemal antibody absorption (FTA-ABS) test and the microhemagglutination *Treponema pallidum* (MHA-TP) test.

Both the RPR and VDRL tests, although relatively easy to perform and inexpensive, lack specificity and cannot be automated. The FTA-ABS and MHA-TP tests are technically more difficult to perform and more expensive. Like the RPR and VDRL tests, these tests require subjective interpretation and cannot be automated.

A Food and Drug Administration-approved enzyme immunoassay (EIA) (CAPTIA Syphilis-G; Trinity Biotech, Jamestown, N.Y.) has recently become available. This test, which assesses immunoglobulin G (IgG) antibodies specific to *T. pallidum*, has the potential to be automated and produces an objective result. Moreover, as the test detects treponema-specific antibodies, it can be used as a stand-alone test.

The objective of the present study was to compare the accuracy of the CAPTIA Syphilis-G EIA to that of a treponema-specific test currently used in our laboratory, the FTA-ABS test (Zeus Scientific, Inc., Raritan, N.J.). Eighty-nine serum samples submitted to our laboratory for syphilis testing were evaluated by both of these test methods.

MATERIALS AND METHODS

Specimens. Eighty-nine stored sera from individual patients previously tested by the FTA-ABS IgG test were evaluated by the CAPTIA Syphilis-G EIA. Forty-one of these samples had previously tested positive by the FTA-ABS IgG test and 48 of these sera had previously tested negative by this method. Some, but not all, of these samples had also been tested with the rapid plasma reagin (RPR) test (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Both the FTA-ABS IgG and RPR tests were performed according to the manufacturers' directions. The degree of positivity for the FTA-ABS IgG test was compared to that for controls and was based on the degree of fluorescence; it was recorded as either nonreactive, minimally reactive, or reactive. Reactive samples were further described as reactive, 1+, 2+, 3+, or 4+, depending on the intensity of the fluorescence. In cases where both the RPR and FTA-ABS IgG tests were performed, technologists performing the tests were not blinded to the results of RPR testing, which was usually performed prior to FTA-ABS IgG testing.

CAPTIA EIA testing. The CAPTIA Syphilis-G EIA was performed according to the manufacturer's directions. Briefly, 10 μ l of serum from each patient and the controls contained in the kit (one negative, one weakly positive, and one strongly positive control) were pipetted into microtiter wells coated with *T. pallidum* antigen and containing 100 μ l of diluent. Following incubation at 37°C for 60 min, the wells were washed with a solution containing 0.05% Tween 20 in phosphate-buffered saline (pH 7.0 to 7.2). Monoclonal antibody (anti-human IgG) labeled with biotin and a streptavidin-horseradish peroxidase conjugate were added. The microtiter plate was incubated at 37°C for 60 min, microtiter wells were washed, tetramethylbenzidine substrate was added to each well, and the plate was then incubated at room temperature for 30 min. Absorbance values

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TABLE 1. Results of the CAPTIA Syphilis-G assay compared with the FTA-ABS test

CAPTIA result	No. of samples with:			
	FTA-ABS result		Discordant result reconciled by repeated FTA-ABS testing (blinded) and/or chart review	
	Reactive	Nonreactive	Reactive	Nonreactive
Reactive	29	1	29	1 ^c
Nonreactive	12 ^a	47	1 ^b	58

^a Nine of the 12 samples were interpreted as minimally reactive; 7 of these 9 were interpreted as nonreactive by repeated FTA-ABS testing (blinded). One of the 12 samples was interpreted as reactive, 2+; this sample was interpreted as nonreactive by repeated FTA-ABS testing (blinded). For 3 of the 12 with repeatedly reactive results for FTA-ABS (blinded and not blinded), the patient had no evidence of clinical syphilis as determined by chart review.

^b This patient had chronic thrombocytopenia which was felt to be part of an undefined autoimmune disorder (see the text). For this patient, the FTA-ABS test was minimally reactive; the MHA-TP test was negative.

^c This patient was not felt to have active syphilis but could have had treated syphilis.

were read at 450 nm with a microtitration plate reader. Antibody indices were calculated by dividing the test sample absorbances by the absorbance of the weakly positive standard. A positive result (as defined by the manufacturer) was an antibody index of >0.900. For the present study, we arbitrarily chose to retest samples with antibody indices of ≥ 0.650 and ≤ 0.900 . If the second (repeated) analysis resulted in an index of >0.900, the sample was considered positive. Six of 89 (6.7%) of samples fell into this range, and therefore the CAPTIA EIA was repeated on these samples.

Resolution of discordant results. Discrepancies between the FTA-ABS IgG and the CAPTIA Syphilis-G assay results were resolved by repeated CAPTIA EIA testing and repeated FTA-ABS IgG testing. In the second FTA-ABS IgG analysis, the technologist was blinded to previous FTA-ABS IgG and RPR results. If discordant results persisted, specimens were also tested by the MHA-TP assay (Bayer Corporation, Diagnostics Division, Elkhart, Ind.) and patient chart reviews were conducted by an infectious-disease specialist, F.R.C., to determine whether there was clinical evidence of syphilis. The MHA-TP assay was performed according to the manufacturer's specifications.

RESULTS

Thirteen of the 89 (15%) samples had discrepant results. Compared to the FTA-ABS assay, the CAPTIA EIA had a sensitivity and specificity and positive and negative predictive values of 70.7, 97.9, 96.7, and 79.7%, respectively (Table 1). In another analysis, discrepancies between results were resolved by repeated CAPTIA EIA testing, repeated FTA testing (technologists were blinded to previous FTA and RPR results), and patient chart reviews. All results of repeated CAPTIA testing were the same as initial CAPTIA results. Seven CAPTIA-negative samples which had previously been interpreted (unblinded) as minimally reactive by the FTA method were subsequently interpreted (blinded) as nonreactive. One other discrepant sample (CAPTIA negative and FTA positive, 2+, unblinded) was FTA negative with repeated testing (blinded).

For the five remaining discrepant samples, chart reviews indicated that one patient (CAPTIA negative and FTA positive [minimally reactive], blinded) had possible syphilis. These five samples were also evaluated and found to be negative by the MHA-TP assay. The patient, who was thought to have possible syphilis, was a 38-year-old male with a history of chronic thrombocytopenia which was felt to be part of an undefined autoimmune disorder. He had no physical signs of syphilis. The physician treating the patient could not determine whether his autoimmune disorder was related to syphilis or whether he had false-positive syphilis serology associated with

the immune disorder. Nevertheless, he was treated for syphilis. Therefore, after repeated testing and chart reviews, 2 of the 89 (2%) samples had discrepant results; the adjusted sensitivity, specificity, and positive and negative predictive values were 96.7, 98.3, 96.7, and 98.3%, respectively.

DISCUSSION

Our results indicate that the CAPTIA Syphilis-G EIA is a reliable method for detecting antibodies specific to *T. pallidum*. We also discovered that for some samples, the FTA-ABS results may have been overinterpreted by technologists. These were often of weak positivity (interpreted as minimally reactive); furthermore, for these samples, RPR results were positive and known to technologists performing the FTA-ABS confirmation test. Repeated FTA-ABS testing was performed in a blinded fashion, and 8 of 12 samples were interpreted as nonreactive. This observation emphasizes the potential problem of subjective interpretation of test results, which is obviated by the CAPTIA Syphilis-G EIA procedure.

The results of our study were similar to those of two other studies recently published. The sensitivity and specificity of the CAPTIA Syphilis-G EIA compared to standard syphilis tests were reported by Hooper and colleagues (1) to be 100 and 99.9%, respectively, and by Reisner and associates (2) to be 100 and 99.8%, respectively. Another EIA developed by a different company also appears to be quite reliable. Zrein and colleagues (4) recently reported that another EIA, which uses two major *T. pallidum* recombinant antigens (the CAPTIA test uses purified *T. pallidum* antigens), had a sensitivity of 100% and a specificity of 99.8% compared to standard syphilis tests. Young and colleagues (3) have compared a novel immunocapture EIA (ICE Syphilis; Murex Diagnostics, Dartford, United Kingdom) to the CAPTIA EIA. The ICE EIA uses three recombinant *T. pallidum* antigens. In that study, the ICE assay detected more treponemal infections in patients coinfecting with human immunodeficiency virus than did the CAPTIA Syphilis-G EIA.

The CAPTIA Syphilis-G EIA may give positive results for patients with active, treated, or inactive disease. Therefore, to assess activity, the manufacturer recommends performing either a nontreponemal test, such as RPR, or the CAPTIA Syphilis-M test. As the CAPTIA Syphilis-M test may give positive results for up to 2 years after successful treatment, it is recommended that nontreponemal titers, for example, RRR titers, be monitored because they may indicate treatment success before IgM titers become undetectable. Although large-scale studies have not been conducted to assess the performance characteristics of the CAPTIA Syphilis-M test, it may be useful to incorporate this along with the CAPTIA Syphilis-G test as a screen, and if either gives a positive result a nontreponemal test can be performed.

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