

## Evaluation of a *Treponema pallidum* Enzyme Immunoassay as a Screening Test for Syphilis

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The CAPTIA Syphilis-G enzyme immunoassay for the detection of antibodies to *Treponema pallidum* was evaluated as a screening test for syphilis in comparison with the standard rapid plasma reagin (RPR) test. One thousand samples were tested, and the standard fluorescent treponemal antibody absorption test and the standard microhemagglutination test were used to confirm the presence of treponemal antibodies. Diagnosis of syphilis was based on traditional standard serology results. Clinical data used in the diagnosis of patients whose samples yielded conflicting results were provided by physicians. Initially, 7 patients whose samples were reactive in the RPR test and 14 patients whose samples yielded positive or equivocal results in the CAPTIA Syphilis-G test were diagnosed as not being infected. After discrepancies due to technical problems were reconciled, samples from six patients remained reactive in the RPR test and that from one patient remained positive in the CAPTIA Syphilis-G test. In addition, seven patients whose samples were nonreactive in the RPR test and two patients whose samples were negative in the CAPTIA Syphilis-G test were diagnosed as having untreated syphilis. After discrepancies were reconciled, samples from five patients remained nonreactive in the RPR test and none remained negative in the CAPTIA Syphilis-G test. Final results indicate that the sensitivities of the RPR test and the CAPTIA Syphilis-G test are 86.1 and 100%, respectively, and that the specificities are 99.4 and 99.9%, respectively. In addition to the improved sensitivity and specificity of the CAPTIA Syphilis-G screen, other potential benefits of this assay lead us to believe that this method could serve as a better screening tool than the RPR test.

Since the mid-1980s, the number of reported cases of syphilis has been on the rise in the United States. Despite the dramatic decline seen after the introduction of penicillin in the 1940s, the number of cases reported in recent years exceeds the national objective for the year 2000 (27). The increased use of cocaine linked with more frequent sexual contact, the exchange of sex for drugs, the possibility of transmission through needle sharing, and the difficulty in tracing the sexual partners of drug users are contributing factors in the spread of the disease (10, 24). The increased numbers of cases among heterosexual men and women have created an increased number of cases of congenital syphilis as well (27). If people such as drug users hesitate to seek medical care and if access to medical care is limited, the need to find a solution to this national health problem will continue to grow (10, 24).

The use of serological tests for syphilis plays an important role in the management of disease transmission. Screening tests for syphilis have been available since the early 1900s. The increase in the number of cases over the past few years has focused attention on the development of new and improved methods such as the enzyme immunoassay for detecting antibodies to *Treponema pallidum*. This method was first described in 1975 by Veldkamp and Visser, who concluded that it might be used in the future as a screening test (29). Since that time, various versions of the assay have been evaluated in Europe and the United States, and opinions about its use as a screening tool have varied (4, 7, 8, 16, 21, 23, 32). Because of the importance of syphilis screening in the management of this epidemic, we chose to evaluate the CAPTIA Syphilis-G enzyme immunoassay as an alternate screening test for laboratories in the United States.

**Sample collection.** For this study we chose 1,000 consecutive serum samples which were submitted to Maryland Medical Laboratory, Inc., Baltimore, Md., for routine syphilis screening by the rapid plasma reagin (RPR) test. Samples were kept refrigerated at 2 to 8°C as several runs of the CAPTIA Syphilis-G assay were performed. Patients were diagnosed as having had syphilis at some time if both the cardiolipin antibody test results and the standard treponemal antibody test results were positive. Clinical data concerning samples yielding negative cardiolipin antibody results accompanied by positive treponemal antibody results were provided by physicians. On the basis of the final data from this study, 34 patients were diagnosed as having had syphilis at some time, while 961 were considered not infected and five diagnoses were inconclusive because serology results varied or patients could not be located for follow-up.

**RPR test.** All samples were tested by the standard RPR test (Becton Dickinson, Cockeysville, Md.) according to the manufacturer's instructions.

**CAPTIA Syphilis-G assay.** All samples were tested in the CAPTIA Syphilis-G assay (BioWhittaker Inc., Walkersville, Md.) according to the manufacturer's instructions. This enzyme immunoassay uses a microplate coated with a sonicate of *T. pallidum*. Serum dilutions of 1:21 were made by using an automated fluid sample handler (Diamedix Corporation, Miami, Fla.). Volumes (100 µl) of the low-positive control (in duplicate), the high-positive control (single), the negative control (single), and diluted samples (single) were transferred to the coated plate and allowed to incubate at 37°C for 1 h. The microplate was then washed by using an automatic microplate washer (Molecular Devices, Menlo Park, Calif.). After the microplate was washed, 100 µl of a biotinylated anti-human immunoglobulin G (IgG) monoclonal antibody labelled with streptavidin-conjugated horseradish peroxidase was added to each microwell. After a 1-h, 37°C incubation, the microplate

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TABLE 1. Results of the CAPTIA Syphilis-G screen versus those of the traditional RPR screen after testing 1,000 serum samples

CAPTIA Syphilis-G result	No. of samples with indicated RPR result			
	Initial screening <sup>a</sup>		Final screening <sup>b</sup>	
	Positive	Negative	Positive	Negative
Positive	20	18	23	9
Equivocal	2	10	3	4
Negative	10	940	7	954

<sup>a</sup> Screen results after initial testing.

<sup>b</sup> Screen results after discrepancies due to technical problems were reconciled.

was washed to remove any unbound material. The amount of bound enzyme was then determined following the addition of 100  $\mu$ l of tetramethylbenzidine chromogen to each well. After a 30-min, room temperature (18 to 25°C) incubation, the reaction was stopped with the addition of 25  $\mu$ l of 2 M sulfuric acid to each microwell. The plate was immediately read on a spectrophotometer at 450 nm, and absorbance readings were recorded. The run was validated according to the manufacturer's protocol for control absorbance readings, which states that the negative control absorbance should be less than or equal to 0.25, the high-positive control absorbance should be greater than or equal to 0.8, and the mean absorbance of the low-positive control should be greater than or equal to 1.5 times the absorbance of the negative control and less than or equal to 0.5 times the absorbance of the high-positive control. Patient sample absorbances were divided by the mean absorbance of the low-positive control to provide index values. Samples with index values in the range of 0.9 to 1.1 (i.e., absorbance within 10% of the absorbance of the low-positive control) were considered equivocal. Samples with values below 0.9 were considered negative, and samples with values above 1.1 were considered positive for IgG antibodies to *T. pallidum*.

**Additional test methods.** All reactive RPR samples and positive or equivocal CAPTIA Syphilis-G samples were tested by the standard fluorescent treponemal antibody absorption (FTA-ABS) test (Zeus Scientific Inc., Raritan, N.J.) according to the manufacturer's instructions. Additional confirmatory testing was performed by use of the standard microhemagglutination (MHA-TP) test (Sera-Tek; Miles Inc., Elkhart, Ind.) according to the manufacturer's instructions. In addition, selected samples were tested for IgM antibodies to *T. pallidum* by use of the CAPTIA Syphilis-M IgM capture enzyme immunoassay (BioWhittaker Inc.) according to the manufacturer's instructions. These selected samples were also tested for IgM antibodies to *T. pallidum* by use of the FTA-ABS 19S IgM assay essentially according to the procedure described in a publication from the National Center for Infectious Diseases of the Centers for Disease Control and Prevention (28). Fractionation of the serum to remove IgG in this procedure was accomplished by use of the Mini Rapi-Sep-M column (Integrated Diagnostics, Baltimore, Md.).

In our study, the CAPTIA Syphilis-G enzyme immunoassay for IgG treponemal antibodies was used to test 1,000 consecutive serum samples that were submitted for screening by the RPR test. Results of both screening methods are displayed in Table 1. Samples with positive or equivocal results in either assay were tested for treponemal antibodies by using the FTA-ABS assay. Minimally reactive FTA-ABS samples and equivocal CAPTIA Syphilis-G samples were also tested by using the MHA-TP assay to further verify the presence or absence of treponemal antibodies.

Of the samples tested, 32 were reactive in the RPR test. Of these 32 samples, 7 were not reactive in the FTA-ABS test or the MHA-TP test. When the tests were repeated, one sample was nonreactive in the RPR test, but six remained reactive and were therefore considered false positives. Another of the 32 reactive RPR samples was minimally reactive in the FTA-ABS test and reactive once and nonreactive once in the MHA-TP test when this test was performed twice. This sample provided inconclusive serology results and therefore an inconclusive diagnosis.

The six false-positive RPR results focus our attention on a problem with the RPR screen. The specificity of cardiolipin assays has been shown to be lower than the specificity of treponemal assays (13). Biological false positives in cardiolipin tests are believed to occur in 1 to 2% of the general population (14). Some reports have shown higher rates (22), but the rate depends on the population studied. If the population includes patients with other treponemal diseases (3), autoimmune disorders such as systemic lupus erythematosus (5, 17, 25), drug addiction (12, 20, 25), genital herpes (20, 30), or leprosy (20, 25), or if there are elderly (9, 20, 25) or pregnant (2, 18, 20, 26) patients, the likelihood of a false-positive cardiolipin test result increases. If a screening method with higher specificity were available, false-positive results would be minimized. As our final results show, the treponemal enzyme immunoassay screen has this quality.

In our study, 15 samples were nonreactive in the RPR test, yielded positive or equivocal CAPTIA Syphilis-G results, and were reactive or minimally reactive in the FTA-ABS test. Upon repeat testing, two samples became reactive in the RPR because of a difference in technical interpretation. There were 13 samples that remained nonreactive in the RPR test upon repeat testing. One sample was equivocal in the CAPTIA Syphilis-G assay, minimally reactive in the FTA-ABS test, and inconclusive in the MHA-TP test because of nonspecific agglutination with unsensitized cells. Because of the serology results with this sample, the patient's diagnosis was considered inconclusive. A second sample, which was nonreactive in the RPR test, positive in the CAPTIA Syphilis-G assay, minimally reactive in the FTA-ABS test, and nonreactive in the MHA-TP test, was of insufficient quantity for further investigation. Because the sample was initially run adjacent to a positive sample in the CAPTIA Syphilis-G assay and the possibility of carryover could not be disproved, the results for this sample were considered incomplete and the patient's diagnosis was considered inconclusive. Of the 11 remaining samples that were nonreactive in the RPR test and whose results were unconfirmed by standard treponemal antibody tests, 10 were diluted and found to have no RPR prozone reactivity. The other sample was of insufficient quantity to be tested for the prozone effect.

The prozone phenomenon occurs in particle agglutination as a consequence of an imbalance between antigen and antibody concentrations. Incomplete cross-linking of particles yields false-negative results. Although our population of patient samples did not reveal any patients with prozone, it is well known that up to 2% of the sera from patients with secondary syphilis yield false-negative RPR results due to the prozone phenomenon (14). Because treponemal antibody tests are not subject to this phenomenon, the treponemal enzyme immunoassay could be more helpful in such situations by providing a positive result (32).

The 11 samples that indicated the presence of IgG treponemal antibodies in the absence of cardiolipin antibodies were tested for the presence of IgM antibodies to *T. pallidum*. The presence of IgM treponemal antibodies has been found in

early primary syphilis. The CAPTIA Syphilis-M assay and the FTA-ABS 19S IgM assay were used to provide additional information for the diagnoses of these patients. Of the 11 samples tested, none clearly indicated the presence of IgM treponemal antibodies in either assay. On the basis of the serology results of the RPR, FTA-ABS, and MHA-TP tests and information provided by physicians, two patients were diagnosed with active syphilis, three were diagnosed with latent syphilis and three were determined to have been treated for syphilis in the past. Two patients could not be located for follow-up, and the remaining patient was diagnosed as having false-positive results in all three treponemal tests because of pregnancy.

Of the 1,000 study samples, 50 yielded positive or equivocal results when screened by the CAPTIA Syphilis-G assay. Two of these samples were from patients with inconclusive diagnoses as described above. The results for 35 samples were confirmed by reactive FTA-ABS and/or MHA-TP results. The results for the remaining 13 samples (7 positive and 6 equivocal) were not confirmed by the standard treponemal tests. Upon repeat testing in the CAPTIA Syphilis-G assay, these 13 samples with unconfirmed results were determined to be negative. We are currently investigating the possibility that the instrument used in diluting these 13 samples was subject to sampling error or carryover. Until the source of this problem can be identified, repeat testing of all positive samples is necessary.

We also recommend testing positive and equivocal CAPTIA Syphilis-G samples with the RPR titer test or another cardiolipin test. Because treponemal antibodies usually remain reactive for life, completing the serology profile with the RPR titer is helpful in determining the stage of disease and monitoring the disease. For example, the presence of treponemal antibodies in the absence of cardiolipin antibodies could indicate latent syphilis. Levels of cardiolipin antibodies decline during latent syphilis, and samples from 20 to 25% of patients become nonreactive in cardiolipin tests (3, 9, 15). The presence of treponemal antibodies in the absence of cardiolipin antibodies may also indicate primary syphilis (1, 6, 9, 11, 20). The ability of the IgG enzyme immunoassay to detect treponemal antibodies in primary syphilis sooner than cardiolipin tests has also been documented (21, 32). Our suggested testing protocol of follow-up testing with the RPR test would provide important information to physicians for diagnosis and treatment as well as to health departments for purposes of contact tracing.

Of the 1,000 study samples, 1 sample yielded a negative CAPTIA Syphilis-G result but was reactive once and nonreactive once in the MHA-TP test when this test was performed twice. As described above, this patient's diagnosis was considered inconclusive. Two study samples produced negative results in the CAPTIA Syphilis-G assay and were reactive in the RPR and FTA-ABS tests. These two samples were assayed again in the CAPTIA Syphilis-G test. One result changed from an index of 0.8 to an index of 0.9-equivocal, suggesting a difference due to interrun variation. The second result changed from an index of 0.3 to an index of 1.3-positive. The difference observed in this result may have been a consequence of an instrument-related error in sampling or an inadequately coated microwell. The index of 1.3 was considered more reliable since it was confirmed by the standard treponemal antibody tests.

Displayed in Table 2 is a summary of the diagnoses and screen results for the 1,000 study samples. After discrepancies due to technical problems were reconciled, fewer false positives and false negatives were found in the CAPTIA Syphilis-G assay.

Although current standard treponemal antibody tests are considered very sensitive and very specific, historically they

TABLE 2. CAPTIA Syphilis-G and RPR screen results in relation to syphilis diagnosis

Screen and result	No. of samples from patients with indicated syphilis diagnosis					
	Initial screening <sup>a</sup>			Final screening <sup>b</sup>		
	+	-	? <sup>c</sup>	+	-	? <sup>c</sup>
<b>RPR</b>						
Positive	24	7	1	26	6	1
Negative	10 <sup>d</sup>	954	4	8 <sup>d</sup>	955	4
<b>CAPTIA</b>						
Positive	29 <sup>e</sup>	8	1	29 <sup>e</sup>	1	2
Equivocal	3	6	3	5 <sup>g</sup>	0	2
Negative	2	947	1	0	960	1

<sup>a</sup> Screen results after initial testing.  
<sup>b</sup> Screen results after discrepancies due to technical problems were reconciled.  
<sup>c</sup> Inconclusive diagnosis due to variable results or inability to locate patients for follow-up.  
<sup>d</sup> Includes three patients who had previously been treated for syphilis; negative results were expected.  
<sup>e</sup> Includes three patients who had previously been treated for syphilis; positive results were expected.  
<sup>f</sup> Includes two patients who had previously been treated for syphilis.  
<sup>g</sup> Includes one patient who had previously been treated for syphilis.

have not been considered for use as screening tests because of their cost, their inability to distinguish disease stages, and their inability to monitor treatment. However, the RPR test was developed as a screening test to provide a sensitive, cost-effective, quick test for field use. The treponemal enzyme immunoassay could achieve these same goals.

First, the sensitivities and specificities of the CAPTIA Syphilis-G and RPR assays in detecting untreated cases of syphilis were calculated. Samples from 3 treated patients yielded negative RPR results and positive or equivocal CAPTIA Syphilis-G results. These contradictory results are understandable and were therefore omitted from the calculations. Table 3 indicates that the CAPTIA Syphilis-G test is more sensitive than the RPR test in detecting untreated cases of syphilis. Our results support the conclusions from other studies in which the CAPTIA Syphilis-G assay was found to be very sensitive and specific for treponemal antibodies (16, 31).

Second, the potential for automation makes the treponemal antibody enzyme immunoassay a more objective test and provides rapid results for batch testing in high-volume laboratories. While our laboratory could experience a cost benefit due to a high volume of samples with a relatively low positivity rate, clinics and large hospital laboratories in which a higher positivity rate exists may not experience such a benefit. How-

TABLE 3. Sensitivity and specificity of CAPTIA Syphilis-G and RPR assays in detecting untreated cases of syphilis<sup>a</sup>

Assay	Initial screening <sup>b</sup>		Final screening <sup>c</sup>	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
CAPTIA Syphilis-G	93.9	98.6	100	99.9
RPR	81.6	99.3	86.1	99.4

<sup>a</sup> Results for patients with inconclusive diagnoses and treated syphilis were not included in calculations.  
<sup>b</sup> Screen results after initial testing.  
<sup>c</sup> Screen results after discrepancies due to technical problems were reconciled.

ever, if this treponemal enzyme immunoassay were to be used as a screening test, it could eliminate the need for confirmation by other treponemal tests. In addition, the improved sensitivity of the assay, as seen in our study, could enable it to detect new cases that would otherwise be missed by the cardiolipin screen. Together these benefits would provide savings in health care expenses and contribute to the prevention of the spread of the disease. Such long-term benefits could certainly outweigh the additional costs which clinics may encounter in purchasing reagents.

Finally, because of its sensitivity and specificity, using the assay as a screening test in the field would also provide many benefits. Many enzyme immunoassays have been modified to rapid-membrane formats that require little or no instrumentation. This modification would be very useful for field testing. A recent study involved initiating a program of screening drug users in high-risk areas such as crack houses and drug sale areas (19). This program was successful in detecting new cases, but some patients could not be located for follow-up examination and treatment despite the short period between the time of phlebotomy and the time at which results were obtained. The study demonstrated that the best approach would be to perform a test on-site and obtain a result immediately. Therefore, a quick, portable treponemal screening test would be very beneficial. The membrane format of testing has already been adopted in many areas of infectious disease serology. We believe that it is realistic to predict that such a method will soon be developed for syphilis serology as well.

A review of our study's results and other potential benefits leads us to consider the CAPTIA Syphilis-G enzyme immunoassay for use as a possible alternate screening test, should the test achieve standard status by the Centers for Disease Control and Prevention. With promising new syphilis tests being developed, we find that it is time for laboratories in the United States to consider improving screening tests and testing protocols. With numbers of syphilis cases remaining higher than our national goal, laboratories need to provide the best screening tests possible to help curb the spread of this infectious disease.

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