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Performance of CAPTIA SelectSyph-G Enzyme-Linked Immunosorbent Assay in Syphilis Testing of a High-Risk Population: Analysis of Discordant Results▽†

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Using the Captia Select Syph-G enzyme-linked immunosorbent assay (ELISA), we analyzed the sera of 1,771 patients from a high-risk population attending sexually transmitted disease (STD) clinics. We focused on discrepancies between the results of the immunoglobulin G (IgG) ELISA and the *Treponema pallidum* hemagglutination (TPHA) test. We identified 57 patients (3.22%) with conflicting results in the IgG ELISA and TPHA test. In order to resolve these discrepancies, these patients' health records were reviewed and additional serological tests (rapid plasma reagin, IgM ELISA, fluorescent treponemal antibody absorption, and Western blotting) were performed. We subsequently diagnosed 22 of these 57 (38.6%) patients with late latent syphilis. None of the patients with discordant test results was diagnosed with early syphilis. We followed 35 of these 57 patients, analyzing two consecutive serum samples at 3 weeks and at 3 months. Discordant results persisted in 12 (33.3%) patients. We successfully resolved the test result discrepancies for 28 patients (80%) involved in follow-up. Captia SelectSyph-G ELISA showed a sensitivity of 99.0%, a specificity of 98.0%, and positive and negative predictive values of 99.3% and 97.2%, respectively. Based on the results of this study, we conclude that the Captia SelectSyph-G ELISA is a reliable tool for syphilis testing in a high-risk population and recommend the utilization of the Captia SelectSyph-G ELISA as a confirmatory test in at-risk patients.

Since 1990, there has been an upward trend in the incidence of both acquired and congenital syphilis in European countries, especially in Eastern Europe. Indeed, the incidence of syphilis has increased worldwide. In the United Kingdom and other developed countries, the incidence of syphilis has increased due to local outbreaks (14, 17), and a higher incidence of syphilis is clearly associated with urban agglomerates and sexual tourism destinations (17).

Antibody detection by serological assay is regarded as the "gold standard" for diagnosing syphilis and for monitoring treatment efficacy (3). However, the reliability of diagnostic kits in different patient populations is sometimes questionable (12). Recent studies have evaluated the performance of various enzyme-linked immunosorbent assays (ELISAs) in detecting syphilis (2, 8, 9, 12, 16, 18); ELISAs were highly sensitive. However, specificities were assessed on a nonselected population (10, 16, 18), and some authors recommended collecting further data on their specificity and reactivity in patients with late-stage disease or in high-risk populations (12, 13). Thus, evaluation of the efficacy of diagnostic kits in clinical settings is critical.

In order to evaluate the performance of an automated immunoglobulin G (IgG) ELISA for syphilis in a high-risk population, we analyzed the sera of 1,771 sexually transmitted disease (STD) clinic patients who were highly suspected to have syphilis or who were known to have latent-stage disease. We compared the treponeme-specific Captia SelectSyph-G assay with the *Treponema pallidum* hemagglutination (TPHA) test, a standard test that gives fewer false-positive reactions than fluorescent antibody tests, and is consider to be highly sensitive (3, 16). The ELISA can be easily automated and provides objective results. In addition, while the TPHA test is usually less expensive than the ELISA, the TPHA test results are read subjectively and recorded manually (12). Furthermore, there is a trend toward automation whenever possible in order to reduce costs, and the automated tests are typically ELISA-type tests (9). Thus, we sought to compare the results of the IgG ELISA with the results of the TPHA test and to determine the source of any discrepancies.

MATERIALS AND METHODS

Serum samples. Serum samples from 1,771 patients were collected and tested during the years 2001 to 2005. The samples originated from an at-risk population attending several STD clinics. The cohort consisted of patients with clinical symptoms consistent with syphilis, patients with syphilis previously treated (patients with early latent and late latent stages of disease), contacts of patients diagnosed with syphilis, patients with other STDs (gonorrhea, chlamydial infections, and herpes genitalis), and other patients at risk.

Basic histories were known for 1,369 (77.3%) patients. The group comprised 249 refugees from countries with high incidence of syphilis (former Soviet Union countries, east Asia, etc.), of which there were 52 sex workers, 236 prisoners, 85 drug abusers, 176 children of mothers with syphilis, and 214 pregnant women. Technologists performing serological tests were blinded to patient histories.

The samples were submitted for routine syphilis testing and were divided into two aliquots. One aliquot was stored at 8°C for a maximum of 5 days before ELISA, TPHA, and rapid plasma reagin (RPR) tests were performed. The other aliquot was maintained at −20°C for further analysis.

ELISA testing. The Captia SelectSyph-G ELISA (Trinity Biotech) was performed with the EVOLIS automated pipetting and washing system (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions. Briefly, 10 μl of serum from each patient and the controls was diluted 1:21 in

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TABLE 1. Comparison of Captia SelectSyph-G ELISA and TPHA syphilis tests in a high-risk population

ELISA result[a]	No. of TPHA results:		
	Positive	Negative	Total
Positive	1,282	27	1,309
Negative	30	432	462
Total	1,312	459	1,771

[a] Sensitivity, 97.7%; specificity, 94.2%; positive predictive value, 97.9%; negative predictive value, 93.5%.

microtiter wells coated with *T. pallidum* antigen and containing 200 μl of sample diluent. After incubation at 37°C for 30 min, the wells were washed with a solution containing 0.05% Tween 20 and Tris buffer in two five-rinse cycles. Anti-human IgG monoclonal antibody labeled with horseradish peroxidase was added, the microtiter plate was incubated at 37°C for 30 min, and wells were washed. Tetramethylbenzidine substrate was added to each well, and the plate was then incubated at room temperature for 30 min. The reaction was stopped by the addition of sulfuric acid. Absorbance at 450 nm was read with a microwell plate reader blanked on air. Antibody indices were calculated by dividing the patients' sample absorbances by the absorbance for the weakly positive control that is included in the kit. A positive result was an antibody index of >0.900.

TPHA test. The Immuntrep TPHA test (Omega Diagnostics) was performed according to the manufacturer's directions. Briefly, diluent was pipetted into the microtiter plate as follows: 25 μl in row 1, 100 μl in row 2, and 25 μl each in rows 3 and 4. One drop (25 μl) of each sample was added to a separate well in row 1. Using a microtitration dilutor, the contents of the wells of row 1 were mixed and 25 μl from each well of row 1 was transferred into the corresponding well of row 2. This procedure was repeated for rows 2 and 3. The volume from row 3 was discarded. Then, the contents of the wells in row 2 were mixed and one drop was transferred into row 4. The test cells (75 μl) were added to row 4, and the control cells (75 μl) were added to row 3. The plate was covered and incubated at room temperature for 45 min. Agglutination patterns were then examined. In a positive result, agglutinated cells formed a layer over the bottom of the well. In a negative result, nonagglutinated cells formed a compact button in the center of the well.

Resolution of discordant results. The initial testing by Captia SelectSyph-G ELISA and TPHA test was performed under blinded conditions. We identified samples with discordant TPHA and ELISA IgG results, and these samples were retested by another lab technician, who was blinded to the results of the first round of testing. Repeatedly discordant samples were identified and analyzed in detail. Information regarding other infectious diseases (e.g., human immunodeficiency virus [HIV] status, other STDs, and Lyme borreliosis), chronic liver diseases, and any other factors that could influence test results was collected.

Repeatedly equivocal results of ELISA (antibody indices of 0.9 to 1.1) were considered positive. The additional treponemal tests (fluorescent treponemal antibody absorption [FTA-ABS] and Western blotting) were performed, and patients' medical records and personal histories were reviewed. The patients with discordant test results were retested in 3 weeks and in 3 months; additional serological testing was also repeated. Follow-up times of 3 weeks and 3 months were chosen to cover a period from an initial phase of syphilis (full seroconversion is expected in 2 to 3 weeks after onset of clinical symptoms) to the longest theoretical incubation period (the incubation period is usually no longer than 3 months). This scheme was generally applied to all problem samples.

The sensitivity, specificity, positive predictive value, and negative predictive value of the Captia SelectSyph-G ELISA were assessed.

Additional serological tests. The RPR test, IgM ELISA (EIAgen TMPA IgM kit; Adaltis, Italy), IgG and IgM Western blots (*T. pallidum* Marblot Strip Test System; MarDx Diagnostic, Inc.), and the immunofluorescence test (FTA-ABS immunofluorescent antibody assay; Zeus Scientific, Inc.) were performed for all patients with discrepant results. The tests were performed according to the manufacturers ' instructions included in the diagnostic kits.

RESULTS

Of 1,771 patients tested, 1,714 (96.8%) had concordant results in the IgG ELISA and the TPHA test: 1,282 patients were IgG ELISA and TPHA positive (ELISA+/TPHA+), and 432 were IgG ELISA and TPHA negative (ELISA−/TPHA−) (Table 1). Fifty-seven patients (3.2%) had discordant test results. This group (mean age, 53 years) consisted of 34 men and 23 women.

TABLE 2. Clinical and serological details of 12 patients with repeatedly discrepant results

Patient[a]	Result by:		Stage[c]	Treatment	History
	IgG ELISA[b]	TPHA			
1M	1.25	Negative	snd	No	Lyme borreliosis
2M	1.6	Negative	snd	No	Chlamydial urethritis
3M	1.70	Negative	snd	No	Psychosis, allergic dermatitis
4F	0.92	Negative	snd	No	85-yr-old woman
5M	0.99	Negative	snd	No	Psychosis, allergic dermatitis
6M	Negative	1:80	LL	Yes	Allergy on a silicon penile implant
7F	Negative	1:80	LL	Yes	Repeated abortions
8M	Negative	1:80	snd	No	Scabies
9F	Negative	1:80	snd	No	Pregnancy
10M	Negative	1:160	LL	Yes	Unclear
11F	Negative	1:80	LL	No	Myocardiopathy
12M	Negative	1:80	snd	No	Gonorrhea

[a] All patients were RPR negative.
[b] Results given as index of positivity.
[c] snd, syphilis not diagnosed; LL, late latent.

In comparison with the TPHA test, the Captia SelectSyph-G ELISA showed a sensitivity of 97.7%, a specificity of 94.2%, and positive and negative predictive values of 93.5% and 97.9%, respectively.

Discordant results. Twenty-seven of the 57 patients with discordant results were ELISA+/TPHA− (see Table SA and Table SB in the supplemental material), while 30 patients were ELISA−/TPHA+ (see Table SC and Table SD in the supplemental material). Overall, weak reaction intensity predominated: 23 of 27 ELISA+/TPHA− patients had antibody indices of <1.5, and 26 of 30 ELISA−/TPHA+ patients had a TPHA titer of 1:80.

During follow-up, we repeated serological examination twice in 35 (61.4%) of 57 patients. In 23 (65.7%) of these patients, previously discordant results proved to be incidental and test results of repeat samples agreed. Discordant results of the same pattern persisted in 12 (34.3%) patients (Table 2). No patient showed different patterns of discordant results during the follow-up. No patient was HIV positive.

ELISA+/TPHA− patients. Of the 27 ELISA+/TPHA− patients, 20 participated in follow-up. Of these, five (20%) had persistently discordant test results on follow-up. All five patients tested negative in the additional serological assays, and the IgG ELISA was their single positive reaction; one of the patients was diagnosed with chlamydial urethritis and one with Lyme borreliosis. The patient with Lyme disease had discordant test results on four consecutive occasions over an 8-month period.

Of the remaining 15 patients, 8 were diagnosed with late latent syphilis previously treated and had positive additional treponemal tests on follow-up. Two patients repeatedly tested negative on follow-up (one of them was diagnosed with a herpetic ulcer). Five patients were children of mothers with treated latent syphilis (Table 3).

TABLE 3. Analysis of discordant results between the ELISA and TPHA syphilis assays

Medical history	No. of discrepant results:		
	ELISA+/TPHA−	ELISA−/TPHA+	Total
Late latent syphilis	13	9	22
Children of mothers with syphilis[a]	5	4	9
Other infectious disease[b]	3	5	8
Pregnancy	0	1	1
Immunological disorders	3	0	3
Unclear	3	11	14
Total	27	30	57

[a] Children with transplacentally derived antibodies.
[b] Erysipelas, chlamydial urethritis, gonorrhea, herpes genitalis, Lyme disease, and scabies.

TABLE 4. Performance of Captia SelectSyph-G after resolution of discrepant results

ELISA result[a]	No. of syphilis test results:		
	Positive	Negative	Total
Positive	1,300	9	1,309
Negative	13	449	462
Total	1,313	458	1,771

[a] Sensitivity, 99.0%; specificity, 98.0%; positive predictive value, 99.3%; negative predictive value, 97.2%.

ELISA−/TPHA+ patients. Of the 30 ELISA−/TPHA+ patients, 15 were tested during follow-up. Of these, seven (46.6%) had persistently discordant test results on follow-up. Four of these patients had late latent syphilis and had a history of testing positive by ELISA and TPHA test. In our study, discordant results were likely due to both decreasing antibody levels and the different sensitivities of the tests. The remaining three patients tested negative by serological assay on follow-up. One patient was a pregnant woman, one patient suffered from scabies, and one had gonorrhea.

Of the remaining eight ELISA−/TPHA+ patients in the follow-up study, five were diagnosed as suffering from late latent syphilis previously treated and had positive additional treponemal tests (one of them was diagnosed with gonorrhea and hepatitis B). Three patients were children of syphilitic mothers with transplacentally derived antibodies.

Overall, 22 (38.6%) of 57 patients with discrepant test results were diagnosed with late latent syphilis (13 [48.1%] ELISA+/TPHA− patients and nine [30.0%] ELISA−/TPHA+ patients). In repeatedly discordant samples, four (26.6%) ELISA−/TPHA+ patients had late latent syphilis; however, none of the ELISA+/TPHA− patients did. There was no patient with early syphilis among the patients with discordant test results. We resolved the result discrepancies for 28 (80%) of the patients in the follow-up.

Of 22 (38.6%) patients with discordant test results who were not included in the follow-up, the additional tests and medical records were positive regarding syphilis in 5 of 7 ELISA+/TPHA− patients, while ELISA−/TPHA+ patients were negative in 13 of 15 cases. One of the ELISA−/TPHA+ patients suffered from erysipelas and one from Lyme disease at the time of diagnosis.

After resolution of the discrepant samples and assessment of patients' clinical status, the Captia SelectSyph-G ELISA showed a sensitivity of 99.0%, a specificity of 98.0%, and positive and negative predictive values of 99.3% and 97.2%, respectively (Table 4).

DISCUSSION

With the exceptions of FTA-ABS and the Venereal Disease Reference Laboratory (VDRL) test, where biologically false-positive results are often reported, there is little data available about false results in the serological evaluation of syphilis. False-negative results have been reported in HIV-positive patients (16).

In 1991, Ross et al. assessed the role of STDs in producing false-positive reactions in the Captia Syph G enzyme immunoassay. They found no cross-reaction between the Captia Syph G enzyme immunoassay and any specific STD- or rheumatoid factor-positive sera (11).

In 2002, Ooi et al. (7) reported on their study evaluating the implications of an isolated positive (all other syphilis serology is negative) syphilis enzyme immunoassay test in a sexual health clinic population in Australia. There were significantly greater numbers of isolated positive syphilis enzyme immunoassay tests in this population compared to lower-risk populations. In 32% of the 22 cases evaluated (men reporting homosexual contact in particular), the medical histories contained clinical grounds for suspecting that the ELISA was accurate (7). This result is inconsistent with our finding that no patients with an isolated positive ELISA result had ever had syphilis.

It is known that nonsyphilitic treponemes can be reactive in serological tests for syphilis, resulting in false positives. Recently, Marangoni et al. demonstrated that a positive serology against *Treponema denticola* is not a cause of false-positive results in syphilis (5). Nevertheless, none of the serological laboratory methods can distinguish between infections caused by closely related spirochetes (*T. pallidum* subsp. *pertenue*, *T. pallidum* subsp. *endemicum*, and *Treponema carateum*) (6). This feature makes serology-based syphilis assays potentially less specific. However, all of the patients with discrepant results in our study had been living in Central Europe, where nonsyphilitic treponematoses are virtually unknown.

As well, the different sensitivities and specificities of serological reactions used throughout the course of syphilis could result in inconsistent test results. The different features of serological tests can be especially important when testing for syphilis during the primary and late latent stages (3, 16). Results from our study support this assertion: approximately 40% of patients with discordant test results had late latent syphilis. Negative results on RPR, IgM ELISA, and Western blot IgM tests over a 3-month period allowed us to exclude the initial phase of syphilis in problem samples.

We resolved the majority of the cases with discordant test results after testing the second serum samples. Therefore, in most cases the discrepant results can be attributed to a transient anomaly. However, one-fourth of all problem samples were discordant for unclear reasons, and one-third of repeatedly tested patients had persistently discrepant test results. In

these situations, the results of additional serological reactions and medical records were helpful in reaching resolution, emphasizing the importance of cooperation between clinicians and laboratories in solving problem cases.

Use of IgG ELISA as a screening test in combination with other tests, or as a stand-alone test, has been investigated (9, 10, 15). In a study by Pope et al., the results of microhemagglutination assay for *T. pallidum* testing agreed with the results of the Captia Syphilis-G test in 97.7% of results (9). Similarly, in our study, agreement between TPHA results and Captia SelectSyph-G ELISA results reached 96.8%, indicating that the hemagglutination tests are generally more sensitive than the ELISAs. Nevertheless, various authors have confirmed that ELISA-based detection of IgG can replace hemagglutination testing when screening for syphilis (1, 9, 15). However, Lefevre et al. did not recommend the use of ELISA for syphilis screening because the Captia Syphilis-G ELISA failed to detect some patients with untreated primary syphilis (sensitivity of 82%) (4). Surprisingly, in our study, patients with discordant results were not those with primary syphilis: these patients more often had late latent stage disease or were falsely positive. Thus, the Captia SelectSyph-G ELISA should not be used as an independent screening test for syphilis. As an aside, the disagreement between our results and those of Lefevre may suggest that patients in our study were referred to STD clinics late, after the primary manifestation of the disease, which is not desirable.

At-risk patients quite often have associated conditions capable of producing cross-reactions during syphilis testing: for example, hepatitis and other liver diseases, drug abuse, or other STDs. Thus, we assume that in a non-at-risk population, the Captia SelectSyph-G test should be at least as reliable as it is in an at-risk population.

Based on the results of this study, we recommend the use of the SelectSyph-G ELISA in at-risk patients as a confirmatory test. For screening, we recommend a combination of a cardiolipin test and a highly sensitive treponemal test, such as TPHA or FTA-ABS. Very early clinical cases should be managed with caution, and testing should be repeated. When doubt arises, the repeat testing in 3 weeks is usually diagnostic.

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