JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1990, p. 1704–1707
0095-1137/90/081704-04$02.00/0

Vol. 28, No. 8

Evaluation of the Captia Enzyme Immunoassays for Detection of Immunoglobulins G and M to *Treponema pallidum* in Syphilis

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Received 16 November 1989/Accepted 2 May 1990

Two new enzyme-linked immunosorbent assays (ELISA), one for the measurement of immunoglobulin G (IgG) (Captia Syphilis-G) and one for the measurement of IgM (Captia Syphilis-M), were evaluated for detecting antibodies to *Treponema pallidum*. Serum samples from 169 patients, 96 with various stages of untreated syphilis, 63 with treated syphilis, and 10 who were noninfected, were investigated. All sera were also examined by traditional treponemal and cardiolipin tests and by the fluorescent treponemal antibody absorption (FTA-ABS) test for 19S(IgM). The overall sensitivity of Captia Syphilis-G was 98.3%. The IgG ELISA was very sensitive (100%) in all stages of untreated syphilis, except in primary syphilis (82%). In all diagnostic groups of syphilis, the reactivity of Captia Syphilis-M was similar to that of the 19S(IgM) FTA-ABS test, except in reinfections, in which the IgM capture ELISA was less sensitive. False-positive IgM capture ELISA results were not found in the 10 neonates born to mothers adequately treated for syphilis. However, of six serum samples containing rheumatoid factor, two were reactive in the Captia Syphilis-M test but not in the 19S(IgM) FTA-ABS test. This indicated that the specificity of the IgM capture ELISA was not absolute. All serum samples from treated patients were reactive in the IgG ELISA, but only 15 samples were reactive in the IgM capture ELISA, which appeared to be as effective as the 19S(IgM) FTA-ABS test in monitoring the effect of treatment. Simultaneous measurement of IgG and IgM antibodies for *T. pallidum* by the Captia immunoassays appears to be an efficient and simple method for confirming the diagnosis of syphilis as well as for indicating whether active disease is present.

Serological testing for syphilis is an important tool in the prompt and accurate diagnosis of all stages of the disease (27). In France, a screening combination comprising the Venereal Diseases Research Laboratory (VDRL) test and the *Treponema pallidum* hemagglutination assay (TPHA) is widely used. The fluorescent treponemal antibody absorption (FTA-ABS) test is often used as a confirmatory test in samples showing reactivity in other assays. Treatment monitoring is currently done by determination of the decline of either anticardiolipin or, less often, antitreponemal immunoglobulin M (IgM) antibodies (8, 10). Unfortunately, the VDRL test and TPHA combination does not lend itself readily to automation, and the FTA-ABS is laborious and time-consuming.

The enzyme-linked immunosorbent assay (ELISA) for antibody to *T. pallidum* has been shown to offer several advantages over the current tests (4, 18, 25): it is simple and rapid, and it gives results that are read objectively.

In the present study, we report an evaluation of two recently introduced ELISA (Captia Syphilis-G and Captia Syphilis-M; Mercia Diagnostics, Guildford, England) to detect IgG and IgM antibodies to treponemes in patients with treated or untreated syphilis.

MATERIALS AND METHODS

Sera. Sera were obtained from specimens submitted to the Diagnostic Laboratory for routine testing and were stored at −70°C until used. A total of 178 serum samples from patients for whom clinical histories could be obtained were included in the study. Of these patients, 96 were untreated. The samples from 63 patients treated for syphilis were collected at various intervals after the start of treatment. Duplicate serum samples from nine treated patients were tested. In addition, samples from 10 neonates of mothers who were adequately treated for syphilis, without clinical signs indicative of syphilis, were used. All samples were reactive in the TPHA and the FTA-ABS test.

Serological tests. All sera were tested by the VDRL test (BioMérieux, Marcy l'Etoile, France); the TPHA (Fujirebio, Tokyo, Japan), by the instructions of the manufacturers; the FTA-ABS test (24); and the 19S(IgM) FTA-ABS test, after fractionation of the serum by sucrose density gradient ultracentrifugation (8). The conjugate used in FTA-ABS was a rabbit anti-human IgG, IgA, and IgM antiserum or a rabbit anti-human IgM antiserum (Dako Immunoglobulins, Copenhagen, Denmark).

ELISA procedures. The Captia Syphilis-G and the Captia Syphilis-M assays were conducted as directed on the product insert with reagents provided by the manufacturer.

Captia Syphilis-G is an indirect ELISA procedure using test kits that include microtitration plates coated with a sonic extract of *T. pallidum* prepared from Nichols strain organisms, positive and negative control serum samples, dilution buffer, wash buffer, tracer complex, and substrate. A 1 in 20 dilution of serum was obtained by adding 20 μl of serum to 380 μl of dilution buffer, pH 7.0 to 7.2. Samples of diluted serum (100 μl) were then added to designated wells, and the plate was incubated at 37°C for 1 h. After incubation, the wells were aspirated and washed with buffer. Tracer complex (100 μl) comprising biotinylated monoclonal antibody against human IgG and streptavidin conjugated with horseradish peroxidase was added to each well, and the plate was incubated at 37°C for 1 h longer. The wells were then aspirated and washed, and 100 μl of substrate (tetramethyl benzidine in dimethyl sulfoxide) was added to each well. the plate was incubated at room temperature for 30 min, and the

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TABLE 1. Results of syphilis serological tests on sera from 96 patients with untreated syphilis

Syphilis category	No. of serum samples	No. positive by test:				
		VDRL	19S IgM FTA-ABS	Captia Syphilis-G	Captia Syphilis-M	TPHA and FTA-ABS
Primary	17	16	17	14	16	17
Secondary	13	13	13	13	11	13
Early latent	14	12	11	14	9	14
Late latent	33	17	1	33	0	33
Neurosyphilis	3	3	1	3	2	3
Congenital	1	1	1	1	1	1
Reinfection	15	15	13	15	8	15

TABLE 2. Results of syphilis serological test on sera from 63 patients with treated syphilis and 10 noninfected neonates

Patient category	No. of serum samples	No. positive by test:		
		19S IgM FTA-ABS	Captia Syphilis-G	Captia Syphilis-M
TPHA and VDRL test positive	46	18	46	14
TPHA positive, VDRL test negative	26	1	26	1
Noninfected neonates	10	0	10	0

reaction was stopped by adding 25 μl of sulfuric acid (2 mol/liter) to each well.

Captia Syphilis-M is a capture ELISA procedure using comparable test kits, which include microtitration plates coated with anti-human IgM and the other reagents mentioned above. Microtiter plates coated with anti-human IgM were incubated for 1 h at 37°C with 100 μl of serum diluted 1/50 in dilution buffer. After the wells had been emptied and washed with 300 μl of wash buffer, the plates were incubated for 1 h with 100 μl of tracer complex, diluted 1/20. The tracer complex consisted of *T. pallidum* antigens, a biotinylated antiaxial filament monoclonal IgM antibody, and streptavidin conjugated with horseradish peroxidase. The wells were again washed, and 100 μl of substrate was added. The reaction was stopped after 30 min by adding 25 μl of sulfuric acid (2 mol/liter).

Test validation and interpretation were performed as follows. The plates were read on a multiscan reader (Titertek) at 450 nm. The tests results were validated by running negative and low-titer positive kit controls. The run was considered valid if the mean absorbance of the negative control was less than 0.25 and the mean absorbance of the low-titer positive control was more than 1.5 times that of the negative control. The cutoff point between positive and negative was set at the absorbance of the low-titer positive control, according to the manual supplied by the manufacturer. Screen samples showing an absorbance value within about 10% of that of the low-titer positive control were tested again. All sera were screened for rheumatoid factor by a latex agglutination test (Rapitex; Behring Werk, Federal Republic of Germany).

RESULTS

Serum samples from 96 patients with untreated syphilis were tested with the Captia Syphilis assays, and the results were compared with those of the other tests (Table 1). All the sera gave positive results in the TPHA and the FTA-ABS test. The IgG ELISA showed sensitivity comparable to that of these tests at all stages of untreated syphilis, except for primary syphilis: two specimens gave negative results, and one was equivocal. The three serum samples gave positive results in the VDRL test, which was equivocal with another serum at this stage. Retesting these samples did not alter the results. In the late latent syphilis, the sensitivity of the Captia Syphilis-G test was higher than that of the VDRL test. The IgM capture ELISA and the 19S(IgM) FTA-ABS test showed comparable sensitivities at all stages except reinfection. With the IgM capture ELISA, the percentage of reactive sera decreased as the stage of syphilis became more advanced. Whereas there were high sensitivities in sera from patients with primary, secondary, and early latent syphilis, sensitivities were lower in sera from patients with late latent syphilis and with reinfection. All samples gave negative results when tested for the presence of rheumatoid factor, except one from a patient with neurosyphilis. The serum of this patient gave a positive result in the IgM capture ELISA but a negative result in the 19S(IgM) FTA-ABS test.

Table 2 shows the incidence of IgM antibodies to treponemes in 82 serum samples from patients with adequately treated syphilis and from noninfected neonates who had positive treponemal reactions. The patients with treated syphilis were divided into two categories: those with positive treponemal test results and a positive VDRL test reaction, and those with positive treponemal test results only. All sera were reactive in the IgG ELISA. Of the 46 samples from patients with treated syphilis who showed positive VDRL test reactions, 11 were positive with both the 19S(IgM) FTA-ABS test and the IgM capture ELISA, 7 were positive with the FTA-ABS test only, and 3 were positive with the IgM capture ELISA only. Of the 26 samples from treated patients with negative VDRL test reactions, one gave a positive result in the IgM capture ELISA and another gave a positive result in the FTA-ABS test. Rheumatoid factor was found in the sera of five patients; they gave negative results in the 19S(IgM) FTA-ABS test, but one gave a positive result in the IgM capture ELISA.

DISCUSSION

The results of this study indicate that the Captia Syphilis-G assay could be an alternative to other treponemal tests for syphilis antibody. The sensitivity was excellent, and it was comparable to the sensitivity of the TPHA and the FTA-ABS test at all stages of the disease, except in primary syphilis. At this stage, the Captia Syphilis-G assay failed to detect antibodies in the sera of three patients who had positive results with all the other tests. The overall sensitivity of the IgG ELISA for the sera from syphilis patients was 98.3%, only slightly lower than those of the TPHA and the FTA-ABS test (100%). An overall sensitivity of 98.4% has recently been reported in a preliminary evaluation of the Captia Syphilis-G assay (28). But this result from a study of 61 patients with positive serological results (1 with untreated primary syphilis, 36 with treated syphilis, and 24 without accurate syphilis history available) cannot be compared with the results of our study. The sensitivity of the Captia Syphilis-G assay for the 17 serum samples from primary syphilis was, in fact, 82% in our series. Similar findings have been reported with another IgG ELISA (16). Serological

detection of untreated early infection is very important in the control of the disease. In light of our results, the use of the Captia Syphilis-G assay for screening purposes instead of the present combination of the VDRL test and TPHA, as has been suggested (28), does not appear to be suitable. The ability of the IgG ELISA to detect VDRL-negative infection at other stages of the disease is also important, since a large percentage of cases of untreated late syphilis (45% in our study) may come into in this category. This high sensitivity of the Captia Syphilis-G assay and its excellent specificity (as reported by Young et al. [28], who studied 1,260 patients with negative serological tests) are ideally suited to confirming the treponemal nature of serum samples preselected on the basis of VDRL test reactivity. A similar conclusion has been reached for another commercially available IgG ELISA, the Bio-EnzaBead test, which could be used as an alternative confirmatory test (2, 11, 23). In our study, the Captia Syphilis-G assay was reactive with all sera from treated patients. This result confirms that treatment monitoring with the IgG ELISA is not of value, because IgG antibodies to treponemes persist after successful treatment (1).

The significance of IgM in syphilis in the areas of treatment evaluation, early detection, surveillance, and congenital syphilis has been reported (6, 15, 21, 26). A reactive treponemal IgM test in untreated patients is proof of active syphilis (9, 12). Treponeme-specific IgM can most reliably be detected in the 19S(IgM) fraction after its separation from serum. The 19S(IgM) FTA-ABS test, which is highly sensitive and specific for treponemal IgM antibodies in all stages of syphilis, is considered a reference method (9, 12). However, this assay is time-consuming and technically complicated, making a simpler procedure desirable. The results of our study showed that in early stages of syphilis, it could be replaced by the Captia Syphilis-M assay, which is easier to perform and more economical. As reported previously (4, 22, 26), we observed a decrease in IgM antibody as the stage of syphilis increased. The sensitivity of the IgM capture ELISA was 94% for primary syphilis, 85% for secondary syphilis, and 82% for early latent syphilis, compared with that of the 19S(IgM) FTA-ABS test. These results are similar to recently reported sensitivity values for the Captia Syphilis-M test (5). In the case of reinfection, however, our study showed that the sensitivity of the IgM capture ELISA was only 62% of that of the 19S(IgM) FTA-ABS test. Lower sensitivity has been observed in reinfections with another IgM capture ELISA by Pedersen et al. (16). Nonreactive (IgM) FTA-ABS tests with sera from reinfected patients have been also reported (7, 19, 20). This may have been due to the phenomenon of steric competition, since many workers succeeded in detecting these antibodies after separating IgM and IgG antibodies (7, 13, 19, 20). Another explanation for this lack of sensitivity might be the fact that a surplus of IgG can inhibit IgM production in vivo (12). Our study confirms that testing for antitreponemal IgM is useful for detecting active disease early but also that the absence of IgM antibodies to treponemes does not imply that syphilis is absent (8, 16, 22).

Detection of the IgM antibody is also important for follow-up treatment or in surveillance. Any appreciable decrease in IgM antibodies may be interpreted as evidence of therapeutic success (8–10, 15, 21). In our study, the Captia Syphilis-M assay was reactive with only 15 serum samples from 63 treated patients. Therefore, it appears to be as effective as the 19S(IgM) FTA-ABS test in monitoring the effect of treatment response.

The results of the present study indicate that the IgM capture ELISA may be useful for the serodiagnosis of neonatal congenital syphilis. None of the serum samples from the 10 newborns with maternal IgG antibodies were reactive with the Captia Syphilis-M assay. On the other hand, this assay was reactive with the serum from a child who had signs of congenital syphilis. Our findings confirm those reported recently by Pedersen et al. (17) with two other IgM ELISA.

As reported by Naot et al. (14) and by Cerny et al. (3), in any study of IgM the question of false-positive reactions due to rheumatoid factors must be considered. Regarding the specificity of a capture ELISA used to detect IgM, rheumatoid factors can cause false-positive results because, bound to the anti-IgM antibodies coated to the microdilution plate, rheumatoid factor can directly bind an IgG class conjugate (16). In the Captia Syphilis-M assay, a tracer complex is used instead of an IgG class conjugate, which should eliminate the interaction of rheumatoid factor. However, of six serum samples containing rheumatoid factor in our study, two were reactive in the Captia Syphilis-M test but not in the 19S(IgM) FTA-ABS test. This limitation could be overcome by precipitating total serum IgG (13).

In conclusion, the simultaneous measurement of IgG and IgM antibodies for *T. pallidum* by the Captia immunoassays appears to be an efficient method for confirming the serodiagnosis of syphilis as well as for supplying the investigator with additional information for serological evaluation of the patient and the disease. Compared with the FTA-ABS test, the Captia Syphilis assays have several advantages. The tests can easily be automated, and the use of automatic plate readers eliminates the subjectivity of visual reading and the fatigue and eye strain associated with microscopy. Finally, the simultaneous utilization of the two Captia assays should limit the possibility of false-negative or false-positive results which can be observed when only one test is carried out.

LITERATURE CITED

1. **Baker-Zander, S. A., R. E. Roddy, H. H. Handsfield, and S. A. Lukehart.** 1986. IgG and IgM antibody reactivity to antigens of *Treponema pallidum* after treatment of syphilis. Sex. Transm. Dis. **13**:214–220.
2. **Burdash, N. M., K. K. Hinds, J. A. Finnerty, and J. P. Manos.** 1987. Evaluation of the syphilis Bio-EnzaBead assay for detection of treponemal antibody. J. Clin. Microbiol. **25**:808–811.
3. **Cerny, E. H., C. E. Farshy, E. F. Hunter, and S. A. Larsen.** 1985. Rheumatoid factor in syphilis. J. Clin. Microbiol. **22**: 89–94.
4. **Farshy, C. E., E. F. Hunter, S. A. Larsen, and E. H. Cerny.** 1984. Double-conjugate enzyme-linked immunosorbent assay for immunoglobulins G and M against *Treponema pallidum*. J. Clin. Microbiol. **20**:1109–1113.
5. **Ijsselmuiden, O. E., J. J. van der Sluis, A. Mulder, E. Stolz, K. P. Bolton, and R. V. W. van Eijk.** 1989. An IgM capture enzyme-linked immunosorbent assay to detect IgM antibodies to treponemes in patients with syphilis. Genitourin. Med. **65**: 79–83.
6. **Johnston, N. A.** 1972. Neonatal congenital syphilis. Diagnosis by the absorbed fluorescent treponemal antibody (IgM) test. Br. J. Vener. Dis. **48**:464–469.
7. **Lefevre, J. C., G. Larrouy, R. Bauriaud, H. Dabernat, M. Abbal, and M. F. Prere.** 1979. Classes d'immunoglobulines (IgM/IgG) retrouvées dans les sérums de syphilitiques aux différents stades de l'affection (apport du FTA-ABS IgM/IgG). Med. Mal. Infect. **9**:206–211.
8. **Lefevre, J. C., M. F. Prere, M. Abbal, and M. B. Lareng.** 1983. Apport du FTA abs IgM quantitatif aux différents stades de la syphilis avant et après traitement. Ann. Dermatol. Venereol. **110**:425–430.

9. **Luger, A. F. H.** 1987. Serological diagnosis of syphilis: current methods, p. 249–274. *In* H. Young and A. McMillan (ed.), Immunological diagnosis of sexually transmitted diseases. Marcel Dekker, Inc., New York.
10. **Merlin, S., J. Andre, B. Alacoque, and A. Paris-Hamelin.** 1985. Importance of specific IgM antibodies in 116 patients with various stages of syphilis. Genitourin. Med. **61**:82–87.
11. **Moyer, N. P., J. D. Hudson, and W. J. Hausler, Jr.** 1987. Evaluation of the Bio-EnzaBead test for syphilis. J. Clin. Microbiol. **25**:619–623.
12. **Muller, F.** 1986. Specific immunoglobulin M and G antibodies in the rapid diagnosis of human treponemal infections. Diagn. Immunol. **4**:1–9.
13. **Muller, F., M. Moskophidis, and H.-L. Borkhardt.** 1987. Detection of immunoglobulin M antibodies to *Treponema pallidum* in a modified enzyme-linked immunosorbent assay. Eur. J. Clin. Microbiol. **6**:35–39.
14. **Naot, Y., E. V. Barnett, and J. S. Remington.** 1981. Method for avoiding false-positive results occurring in immunoglobulin M enzyme-linked immunosorbent assays due to presence of both rheumatoid factor and antinuclear antibodies. J. Clin. Microbiol. **14**:73–78.
15. **O'Neill, P., and C. S. Nicol.** 1972. IgM class antitreponemal antibody in treated and untreated syphilis. Br. J. Vener. Dis. **48**:460–463.
16. **Pedersen, N. S., C. S. Petersen, and N. H. Axelsen.** 1982. Enzyme-linked immunosorbent assay for detection of immunoglobulin M antibody against the Reiter treponeme flagellum in syphilis. J. Clin. Microbiol. **16**:608–614.
17. **Pedersen, N. S., J. P. Sheller, A. V. Ratnam, and S. K. Hira.** 1989. Enzyme-linked immunosorbent assays for detection of immunoglobulin M to nontreponemal and treponemal antigens for the diagnosis of congenital syphilis. J. Clin. Microbiol. **27**:1835–1840.
18. **Pope, V., E. F. Hunter, and J. C. Feeley.** 1982. Evaluation of the microenzyme-linked immunosorbent assay with *Treponema pallidum* antigen. J. Clin. Microbiol. **15**:630–634.
19. **Sato, T., E. Kubo, M. Yokota, T. Kayashima, and T. Tomizawa.** 1984. *Treponema pallidum* specific IgM haemagglutination test for serodiagnosis of syphilis. Br. J. Vener. Dis. **60**:364–370.
20. **Schmidt, B. L.** 1980. Solid phase hemadsorption: a method for rapid detection of *Treponema pallidum*-specific IgM. Sex. Transm. Dis. **7**:53–58.
21. **Shannon, R., and S. D. Booth.** 1977. The pattern of immunological responses at various stages of syphilis. Br. J. Vener. Dis. **53**:281–286.
22. **Shannon, R., C. G. Copley, and G. D. Morrison.** 1980. Immunological responses in late syphilis. Br. J. Vener. Dis. **56**: 372–376.
23. **Stevens, R. W., and M. E. Schmitt.** 1985. Evaluation of an enzyme-linked immunosorbent assay for treponemal antibody. J. Clin. Microbiol. **21**:399–402.
24. **U.S. Public Health Service.** 1969. Manual of tests for syphilis, p. 15–43. U.S. Government Printing Office publication no. 411. U.S. Government Printing Office, Washington, D.C.
25. **Veldkamp, J., and A. M. Visser.** 1975. Application of the enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of syphilis. Br. J. Vener. Dis. **51**:227–231.
26. **Wilkinson, A. E., and P. Rodin.** 1976. IgM-FTA test in syphilis in adults. Its relation to clinical findings. Br. J. Vener. Dis. **52**:219–223.
27. **World Health Organization.** 1982. Treponemal infections. W.H.O. Tech. Rep. Ser. **674**.
28. **Young, H., A. Moyes, A. McMillan, and D. H. H. Robertson.** 1989. Screening for treponemal infection by a new enzyme immunoassay. Genitourin. Med. **65**:72–78.