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Sodium Desoxycholate-Extracted Treponemal Antigen in an Enzyme-Linked Immunosorbent Assay for Syphilis

ELIZABETH F. HUNTER,[1]* CAROL E. FARSHY,[1] SALLY L. LISKA,[2] DONNA D. CRUCE,[1] JOHN A. CRAWFORD,[1] AND JOHN C. FEELEY[3]

Sexually Transmitted Diseases Laboratory Program,[1] and Division of Bacterial Diseases,[3] Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333, and Department of Health and Rehabilitative Services, Orlando Branch Laboratory, Orlando, Florida 32803[2]

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The extraction of *Treponema pallidum* antigen with sodium desoxycholate, based on a previously described procedure (J. Portnoy and H. J. Magnuson, J. Immunol. 75:348–355, 1955), was used in an enzyme-linked immunosorbent assay (ELISA) test for syphilis. The antigen was prepared from *T. pallidum* street strain no. 14, and its overall sensitivity and specificity was compared with those of sonicated antigen preparations made with phosphate-buffered saline. The optimum serum dilution for testing and the significant absorbance reading at 490 nm were selected by examination of quantitative dilutions of 91 sera from presumably normal individuals and 92 sera from syphilitics. The time and temperature of serum and conjugate incubations were also examined. With an absorbance reading of ≥0.2 at the 1:80 serum dilution, 88 (95.8%) of 92 sera from syphilitics were reactive in the ELISA test with desoxycholate-extracted antigen, and 82 (89.1%) were reactive with the sonicated antigen. Only one nonsyphilitic serum was reactive with each antigen. Greater sensitivity without loss in specificity was obtained with longer serum and conjugate incubations. We concluded that an ELISA test with sodium desoxycholate-extracted antigen is more sensitive than and equally specific to an ELISA with sonicated treponemal antigen.

The use of sonicates of *Treponema pallidum* (6, 8, 9) or the axial filaments of *T. phagedenis* biotype Reiter as antigens in the enzyme-linked immunosorbent assay (ELISA) test for syphilis has been described (P. H. Hardy, E. E. Nell, and A. J. O'Beirne, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C190, p. 308). Acceptable sensitivity and specificity have apparently been obtained with these preparations; however, separation of the serologically active component from *T. pallidum* would be advantageous. An extraction procedure for isolating serologically active *T. pallidum* antigen for the *T. pallidum* complement fixation test was described by Portnoy and Magnuson (7). This procedure involves preliminary extraction of lipid fractions followed by subsequent extraction with sodium desoxycholate (SD).

Recently, there have been reports of an SD extraction procedure for preparing agglutinating antigens from oral spirochetes (2a) and, more recently, for an ELISA test to evaluate dental problems (2b).

Here we describe a *T. pallidum* antigen extraction based on the procedure of Portnoy and Magnuson (7) and compare the overall specificity and sensitivity of this antigen in an ELISA procedure with those of sonicated preparations made with phosphate-buffered saline (PBS).

MATERIALS AND METHODS

Antigen. *T. pallidum* street strain no. 14 was used for antigen preparations. Propagation in adult male rabbits and extraction of the infected testicular tissue generally followed the procedure described for fluorescent treponemal antibody absorption (FTA-ABS) test antigen (2). Treponemes were washed one time in PBS, and the sediment was suspended in PBS containing 0.1% NaN_3. The treponemal suspension was held at 2 to 8°C for a minimum of 1 week. Treponemal yields ranged from 4.4×10^7 to 2.8×10^9 per ml. Ultrasonic treatment for sonicated antigen consisted of three 15-s treatments with a sonifier (Branson Instruments Co.) at 50% output (4).

SD extraction. The SD extraction procedure of Portnoy and Magnuson (7) was modified only in terms of the handling of ether extractions. These extractions were performed under a chemical safety hood, while a temperature below 2°C was maintained with dry ice in a styrofoam container. Treponemes were allowed to settle without centrifugation, and the supernatant fluid was evaporated.

Protein determinations were performed by the Lowry procedure (3). Antigen suspensions were heated for 1 h at 100°C to determine the heat lability of the antigen.

TABLE 1. Comparison of ELISA absorbance values at a low serum dilution when SD antigen was used

Serum type and dilution	No. of sera with absorbance at 490 nm of:			
	≥0.300	0.200 to 0.299	0.100 to 0.199	<0.100
Nonsyphilitic				
1:20	10	12	34	35
1:40	1	6	33	51
1:80	1	0[a]	19	71
Syphilitic				
1:20	91	0	1	0
1:40	87	4	1	0
1:80	80	8	3	1

[a] An absorbance reading of ≥0.200 at 1:80 serum dilution was selected to indicate a reactive test.

TABLE 2. Comparison of ELISA absorbance values at a low serum dilution when sonicated antigen was used

Serum type and dilution	No. of sera with absorbance at 490 nm of:			
	≥0.300	0.200 to 0.299	0.100 to 0.199	<0.100
Nonsyphilitic				
1:20	4	10	49	28
1:40	1	1	35	54
1:80	0	1[a]	19	71
Syphilitic				
1:20	77	12	3	0
1:40	75	13	4	0
1:80	56	26	8	2

[a] An absorbance reading of ≥0.200 at a 1:80 serum dilution was selected to indicate a reactive test.

Procedure. The ELISA test with sonicated or extracted antigen was an indirect procedure, with times and temperature steps based on the procedure for *Toxoplasma* sp. (10) and the test for *Escherichia coli* enterotoxin (11). Sera were diluted serially with Titertek automatic multichannel pipettes in 0.01 M PBS containing 0.05% Tween 20 and 1.0% normal rabbit serum. Serum dilutions were incubated at 2 to 8°C for 16 h, and plates were washed three times in PBS containing 0.05% Tween 20. Peroxidase-labeled anti-human immunoglobulin G (Miles Laboratories, Inc.) was diluted for use in PBS-Tween 20-normal rabbit serum and incubated for 1 h at 37°C. The washing steps were repeated, and orthophenylene diamine was added as the substrate. The reactions were stopped with 8 N H_2SO_4 after 30 min of incubation at approximately 25°C and read with an MR 580 reader (Dynatech Laboratories, Inc.) at 490 nm. A well containing the substrate only was used to zero the machine.

Antigen titration. Antigen suspensions were diluted 1:10 to 1:320 in 0.05 M carbonate buffer, pH 9.6, and plated in 100-μl volumes in Cooke microtiter flat-bottom ELISA plates. Plated antigen was incubated for 3 h in a 37°C water bath followed by 16 h at 2 to 8°C. Plates were held at 2 to 8°C until ready for use. Reactive and nonreactive controls diluted 10^{-1} to 10^{-6} were used to establish optimum antigen dilution.

Normal testicular antigen. Normal testicular tissue was extracted and processed by the methods previously described for treponemal antigen (2, 7). Antigen suspensions were titrated with the reactive and nonreactive control sera.

Serum and conjugate incubations. Reactive and nonreactive control serum dilutions were incubated at 2 to 8°C for 24 h, and the results were compared with those obtained after 2- and 4-h incubations at 37°C. At the same time, conjugate incubations of 30 min and 1 h at 37°C were compared with each other.

Sera. For serum evaluation, sera from patients with clinically documented syphilis or from nonsyphilitics were obtained through the Sexually Transmitted Disease Serum Bank, Centers for Disease Control. All sera from syphilitics were FTA-ABS test (5) reactive. With the exception of one serum which gave a borderline FTA-ABS test result, all sera from nonsyphilitics were nonreactive.

RESULTS

Seven antigen preparations were extracted with SD and evaluated in the ELISA procedure. Antigens plated in Cooke microtiter plates consistently had a titer of ≥1:40. The antigens were stable when stored at −20°C before being plated. Plated antigens were held at 2 to 8°C for up to 5 weeks without showing variation in the strength or specificity of the reaction.

The absorbance readings obtained at serum dilutions of 1:20 through 1:80 were compared after 91 sera from nonsyphilitics and 92 sera from syphilitics were tested with the SD-extracted antigen (hereafter referred to as the SD antigen) in the ELISA test (Table 1). At a 1:80 serum dilution, only one serum in the nonsyphilitic group had an absorbance reading of ≥0.200, whereas 88 (95.6%) of the sera from syphilitics had an absorbance value of ≥0.200.

A similar comparison with the sonicated antigen is shown in Table 2. Again, only one serum from the nonsyphilitic group had an absorbance reading of ≥0.200 at the 1:80 serum dilution, but 82 (89.1%) sera from syphilitics had absorbance values of ≥0.200.

The results of quantitative testing of the 92 sera from syphilitics correlated with stage of syphilis are shown in Table 3. Most sera were obtained from patients with treated syphilis. The ELISA titer was slightly higher when the serum was plated on the SD antigen rather than the sonicated antigen.

The results of ELISA tests performed with various serum and conjugate incubations are shown in Fig. 1 and 2. A onefold-higher serum dilution was obtained with the SD antigen when

TABLE 3. Correlation of ELISA titers for SD and sonicated antigens with stage and treatment status of syphilis[a]

Syphilis stage and treatment status	No. of sera with ELISA titer of:									
	<80		80 to 160		320 to 640		1,280 to 2,560		5,120 to 10,240	
	SD	Sonicated	SD	Sonicated	SD	Sonicated	SD	Sonicated	SD	Sonicated
Primary										
Untreated		1	1					1	1	
Treated	1	2	5	8	7	3		1	1	
Secondary										
Untreated						1	1			
Treated	1	2	3	8	10	11	14	10	4	1
Latent										
Untreated			1	1						
Treated	2	1	4	13	13	10	15	11	1	
Stage										
Unknown	1	1		4	4	1	2	1		

[a] A total of 92 syphilitic sera were examined.

the serum was incubated at 2 to 8°C for 16 h and the conjugate was incubated for 30 min or 1 h. No increase in reactivity was noted in the nonreactive serum. The titer obtained with the sonicated antigen was one- to twofold lower than the titer obtained with the SD antigen. The 16-h serum incubation and 1-h conjugate incubation yielded a twofold-higher titer for the reactive serum, with no reactivity of the nonreactive serum.

When the SD antigen was heated at 100°C for 1 h, a fourfold drop in titer occurred, but activity was not eliminated. Protein determinations for the undiluted antigen ranged from 50 to 120 μg/ml.

In antigen titrations of normal testicular tissue, low-grade reactivity with PBS-extracted antigen was detected when sera were diluted 1:10, but reactivity was not observed when the dilutions were greater. Forty-eight sera from syphilitics and nonsyphilitics were screened on normal testicular antigen at the 1:80 serum dilution. No reactivity was observed with these sera. The protein determination for this antigen at the 1:80 dilution was approximately 1 μg per well, as compared with 0.1 μg per well for the treponemal antigen. No reactivity was observed with normal testicular SD antigen at any serum dilution.

DISCUSSION

An SD antigen, extracted by a modification of the method used by Portnoy and Magnuson (7) for the *T. pallidum* complement fixation test, was used in an ELISA test for syphilis. The antigen was prepared from *T. pallidum* street strain no. 14. As reported by Portnoy and Magnuson, the antigen contains a heat-labile factor and a heat-stable factor.

Although the SD and sonicated antigens were not made from the same starting material, the consistently satisfactory quality of the SD antigens suggests that the procedure is reproducible. In addition, the ELISA reactions obtained with the SD antigen appeared to be stronger and clearer than those obtained with the sonicated antigen. Sensitivity was slightly greater with the SD antigen, and essentially, the SD antigen had the same specificity as did the sonicated antigen. Longer incubation periods for the serum and conjugate increased sensitivity without diminishing specificity.

Quantitative titers were easily obtained with

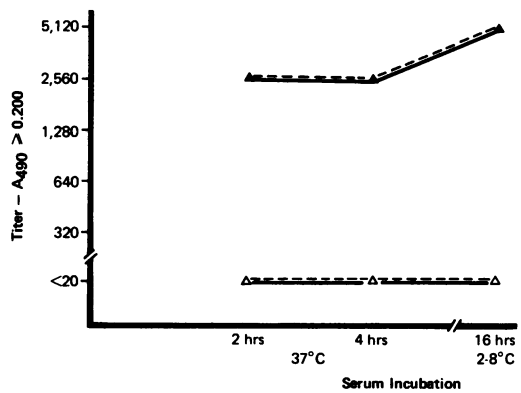

FIG. 1. Comparison of ELISA titers obtained with SD antigen when serum and conjugate incubations were varied. Symbols: ▲, reactive serum; △, nonreactive serum; ---, 30-min conjugate incubation; —, 1-h conjugate incubation.

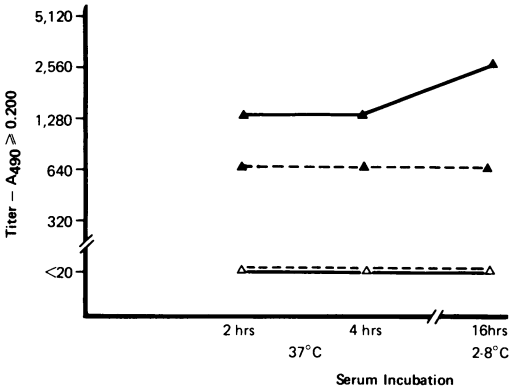

FIG. 2. Comparison of ELISA titers obtained with sonicated antigen when serum and conjugate incubations were varied. Symbols: ▲, Reactive serum; △, nonreactive serum; ---, 30-min conjugate incubation; —, 1-h conjugate incubation.

Titertek automatic multichannel pipettes. The performance of qualitative testing in microtiter plates could present problems. First, it would be more difficult to place the appropriate serum in inside wells and properly identify the serum. In addition, the necessity of preventing wash-over among wells would be as critical in ELISA tests as in immunofluorescence tests (1).

In the ELISA procedure performed on syphilitic sera selected on the basis of FTA-ABS reactivity, sensitivities of 95.7% and 89.1% were obtained for SD and sonicated antigens, respectively. Differences in sensitivity between the FTA-ABS and the ELISA tests were not great, considering that the FTA serum is diluted 1:5 and the recommended serum dilution for the ELISA is 1:80. The sera for the ELISA test were not absorbed to remove group antibody; however, for the number of sera that we used, the ELISA test with both the SD and the sonicated antigens appeared to be comparable in specificity to the FTA-ABS test.

The ELISA procedure has been applied to many areas of microbiology. Since the early work by Veldkamp and Visser (8) and Voller et al. (9), the application of ELISA techniques to syphilis serology has developed slowly, perhaps because specificity has been low or because the antigen suspension has not been stable. Seven antigen preparations were prepared by SD extraction, with similar patterns of activity. Storage of undiluted antigen at −20°C has been satisfactory for at least 4 months, and plated antigen has been satisfactory for up to 5 weeks.

One of the main advantages of the ELISA procedure is that automated microplate readers can be used, thus eliminating visual interpretation. In this study, an absorbance reading of ≥0.200 at 490 nm was considered significant, and a serum dilution of at least 1:80 was necessary for a starting dilution. These recommendations are similar to those previously reported (6; P. H. Hardy, E. E. Nell, and A. J. O'Beirne, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C190, p. 308).

Investigators must consider the possibility that in the future, ELISA procedure will become sufficiently sensitive and specific to replace other treponemal tests.

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