Increased Sensitivity of the Fluorescent Treponemal Antibody Absorption Test with Biotin/Avidin: A Comparison with Conventional FTA-ABS

ELISABETH BERNTSSON

Department of Clinical Bacteriology, University of Gothenburg, Gothenburg, Sweden

Berntsson E. Increased sensitivity of the fluorescent treponemal antibody absorption test with biotin/avidin. A comparison with conventional FTA-ABS. Acta Derm Venereol (Stockh) 1985; 65: 89–91.

The biotin/avidin system was adapted to the FTA technique for detection of IgG, IgM, and IgA antibodies specific to *Treponema pallidum*. By using biotin/avidin IgG antibodies could be detected in a serum dilution 15 000 times higher, and IgM 3 000 times higher than was possible with conventional FTA technique. IgA antibodies, however, were not detected with any of the methods. *Key words: FTA-ABS; Biotin/Avidin.* (Received August 2, 1984.)

Elisabeth Berntsson, Department of Clinical Bacteriology, University of Gothenburg, Guldhedsgatan 10, S-413 46 Gothenburg, Sweden.

Since the introduction of the fluorescent treponemal antibody (FTA) test (1) the method has gone through several modifications, e.g. fluorescent treponemal antibody absorption (FTA-ABS) test (2). In the FTA-ABS test the sera to be tested are absorbed with common treponemal antigen, "sorbent" (3), to increase sensitivity and specificity of the FTA test. The FTA-ABS test is still the most used method for detection of specific antibodies to *Treponema pallidum*. (Henceforth the abbreviation FTA is used instead of FTA-ABS.)

The extraordinary high affinity between the vitamin biotin and avidin has been used in different immunological techniques to increase the sensitivity (4). It was therefore of interest to evaluate the biotin/avidin system in the FTA, and to compare the results with those obtained with conventional FTA. This was the aim of the present experiments.

MATERIAL AND METHODS

Sera

Sera routinely submitted to the laboratory from 34 patients (24 men and 10 women, 1–52 years old, median age 30.5 years) were tested.

Fluorescent treponemal antibody absorption (FTA-ABS) test

Slides with 8 mm diameter circles (Dynatech Laboratories Ltd., Billingshurst, England) were applied with smears of *T. pallidum*, Nichols' strain (bio Mérieux, Charbonnières-les-Bains, France), dried and fixed in acetone for 10 min. Test sera heated at 56°C for 30 min were diluted 1/5, 1/25, 1/125, 1/625, 1/3 125, 1/15 625, 1/78 125, 1/390 625, 1/1 953 125, 1/9 765 625, and 1/48 828 125 in sorbent (bio Mérieux). Each smear was covered with 0.04 ml diluted test serum. The slides were placed in a moist chamber 30 min at 37°C, washed twice in phosphate-buffered saline (PBS), rinsed in distilled water, and dried. After this either conventional FTA technique was followed (I) or a biotin/avidin technique was used (II).

I. *Conventional FTA*

The conventional FTA was performed according to the routine in the laboratory (5). Each smear was covered with 0.04 ml fluorescein labelled anti-human IgG (The National Bacteriological Laboratory, Stockholm, Sweden) diluted 1/100 in PBS, or fluorescein labelled anti-human IgM (Wellcome Research Laboratories, Beckenham, England) diluted 1/50 in PBS, or fluorescein labelled anti-human IgA (Nordic Immunological Laboratories, Tilburg, The Netherlands) diluted 1/50 in PBS. After 30 min at 37°C in a moist chamber the slides were washed twice in PBS, rinsed in distilled water, and dried.

After covering with a coverslip the slides were read in a Zeiss epifluorescence microscope (ocular×10, objective×25).

II. *FTA with biotin/avidin*

Each smear was covered with 0.04 ml biotinylated anti-human IgG (Vector Laboratories, Inc., Burlingame, CA, USA) diluted 1/500 in PBS, or biotinylated anti-human IgM (E-Y Laboratories, San Mateo, CA, USA) diluted 1/500 in PBS, or biotinylated anti-human IgA (E-Y Laboratories) diluted 1/100 in PBS. After 30 min at 37°C in a moist chamber the slides were washed twice in PBS, rinsed in distilled water, and dried. Each smear was then covered with fluorescein-avidin D (Vector Laboratories, Inc.) diluted 1/1 000 in PBS. After 30 min at 37°C the slides were washed twice in PBS, rinsed in distilled water, and dried. After covering with a coverslip the slides were read in a Zeiss epifluorescence microscope (ocular×10, objective×25).

RESULTS AND DISCUSSION

The results of IgG antibody determinations are summarized in Table I and those of IgM antibody determinations in Table II.

All negative sera but one did not differ between the two test systems. This serum showed an IgM antibody titer of 1/25 in biotin/avidin FTA, while conventional FTA was negative. Clinically this patient had a secondary syphilis.

Sera positive in conventional FTA were positive in biotin/avidin FTA and vice versa, except for the one serum mentioned above. With biotin/avidin FTA IgG antibodies could be detected in a serum dilution 15000 times higher and IgM antibodies in a dilution 3000 times higher than with conventional FTA. In accordance with earlier findings IgA antibodies could not be demonstrated by means of any of the test systems (6). It is assumed, that IgA antibodies are no longer reactive, or only present in trace amounts, after adding sorbent to serum. The background fluorescence in the biotin/avidin system was reduced compared to conventional FTA, and the slides could be read much faster and with a higher accuracy than the conventional FTA slides.

In conclusion: with biotin/avidin a striking increase in sensitivity of the FTA test can be achieved in combination with easier read slides.

Table I. *IgG antibodies to T. Pallidum determined with biotin/avidin FTA and conventional FTA*

Figures indicate number of patients (n=34)

Antibody titer: FTA with biotin/ Avidin	Antibody titer: conventional FTA			
	<1/25	1/25	1/125	1/625
<1/25	13			
1/25				
1/125				
1/625		1		
1/3 125		2		
1/15 625		2	7	
1/78 125			2	1
1/390 625			3	2
1/1 953 125				
1/9 765 625				1

Table II. *IgM antibodies to T. pallidum determined with biotin/avidin FTA and conventional FTA.*

Figures indicate number of patients (n=34)

Antibody titer: FTA with biotin/ avidin	Antibody titer: conventional FTA	
	<1/25	1/25
<1/25	24	
1/25	1	
1/125		1
1/625		6
1/2.125		
1/15.625		1
1/78.125		1

REFERENCES

1. Deacon WE, Falcone VH, Harris A. A Fluorescent test for treponemal antibodies. Proc Soc Exp Biol Med 1957; 96: 477–480.
2. Hunter EF, Deacon WE, Meyer PE. An improved FTA test for syphilis, the absorption procedure (FTA-ABS). Public Health Rep 1964; 79: 410–412.
3. Stout GW, Kellogg DS, Falcone VH, McGrew BE, Lewis JS. Preparation and standardization of the sorbent used in fluorescent treponemal antibody-absorption (FTA-ABS) test. Health Laboratory Science 1967; 4: 5–8.
4. Hsu S-M, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981; 29: 577–580.
5. Staff, Venereal Disease Research Laboratory: Technic for the fluorescent treponemal antibody-absorption (FTA-ABS) test. Health Laboratory Science 1968; 5: 23–30.
6. Julian AJ, Logan, LC, Norins LC. Early syphilis: Immunoglobulins reactive in immunofluorescence and other serologic tests. J Immunol 1969; 102: 1250–1259.