AN IMPROVED FTA TEST FOR SYPHILIS, THE ABSORPTION PROCEDURE (FTA-ABS)

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IN THE fluorescent treponemal antibody (FTA) procedure, as in other serologic tests for syphilis, difficulties have been encountered in increasing sensitivity without sacrificing specificity (*1*). Recent findings by immunofluorescent methods suggest that a major cause of nonspecificity in the FTA test may be the occurrence of common or group antigens shared by both pathogenic and saprophytic treponemes. Demonstrations that the corresponding nonspecific antibodies can be removed by appropriate absorption procedures suggested the application of principles that have resulted in an improved FTA test designated as the FTA absorption procedure (FTA-ABS). Its sensitivity and specificity were determined by testing critical categories of selected human serums, using several FTA test procedures and the *Treponema pallidum* immobilization (TPI) test.

Materials and Methods

As available, specific categories of test specimens were obtained from the serum bank of the Venereal Disease Research Laboratory. These consisted of 76 serums taken from documented darkfield positive primary syphilis patients before treatment and 74 serums from persons without clinical or serologic evidence of any previous major treponemal infection. These serums had been used in the Serology Evaluation Research Assembly (SERA) Study of 1956–57 (*3*). In addition 38 serums were included from patients showing reactivity unrelated to syphilis in one or more nontreponemal antigen tests. Careful clinical and laboratory studies were employed in defining these as biologic false positive serums. Eighty-two specimens were included from the 1962 Tuskegee study (*4*, *5*), of which 46 were syphilis serums defined as late-treated, untreated, or inadequately treated syphilis, and 36 were nonsyphilitic controls.

Comparative serologic testing of all serums consisted of the FTA–200 and TPI tests (*6*), the FTA 1–5 procedure performed in the same manner as the FTA–200 except that the test serum is diluted 1:5, and the experimental procedure, the FTA-ABS test.

The experimental procedure was conducted in the following manner. The Reiter treponeme which contains nonspecific common or group antigen was employed to study the feasibility of the absorption approach (*2*). In the present study, a sonicate of this spirochete was used in place of intact Reiter treponemes because of difficulties experienced in microscopic differentiation of this treponeme and *Treponema pallidum*. Reiter treponemes were grown for 96 hours at 37° C. in Difco-NIH thioglycollate broth enriched with heated 10 percent rabbit serum. Intact treponemes were collected by centrifugation, washed three times in phosphate buffered saline, pH 7.2, resuspended in buffered saline, and adjusted to approximately 40 times a MacFarland No. 10 density. Disruption was accomplished by treatment for 1 hour in a Raytheon sonic oscillator, 250 w., 10 kc./second, 0–5° C. Remaining intact treponemes were removed by centrifugation at 9,370 times g. The supernatant fluid was standardized by titration, using a known nonsyphilitic serum demonstrating strong nonspecific reactivity in the FTA 1–5 test. Absorption tests were performed at the

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1:5 dilution of test serum, with the standardized Reiter sorbing agent as a diluent (0.05 ml. inactivated serum plus 0.2 ml. antigen). Simultaneously, similar dilutions were made in buffered saline for the FTA 1–5 test without absorption.

A well-documented nonsyphilitic serum demonstrating nonspecific reactivity in the FTA 1–5 test and a syphilis serum, preferably of low titer as is frequently obtained in primary syphilis, were included in each test run. In addition, a nonspecific staining control consisting of a *T. pallidum* smear and fluorescein-labeled antihuman globulin was examined routinely.

T. pallidum antigen smears were prepared as for the standard FTA procedure (*6*). Commercially available fluorescein-labeled antihuman globulin was employed throughout the study.

Microscopic examinations were made using Leitz ultraviolet equipment with HBO–200 lamp. The filter system consisted of a BG–12, 4 mm. exciter filter and an OG–1 barrier filter.

Results and Discussion

The test findings are compared in the table. The FTA–200 and the TPI tests appear equally effective in detecting syphilis (sensitivity) in the primary syphilis group; 28 serums (36.8 percent) were reactive by both procedures. The problem of sensitivity versus specificity is well illustrated in the FTA 1–5 saline dilution test. Sensitivity was increased to 100 percent in the primary syphilis group, but specificity was significantly decreased, as shown by the occurrence of 19.7 percent reactions in the nonsyphilitic group. By this test, nonspecificity was also indicated in the biologic false positive group (31.6 percent reactors) and in the Tuskegee study control group (33.3 percent reactors). On the other hand, results obtained with the experimental FTA-ABS test are outstanding both in sensitivity and specificity. Here the sensitivity in the primary syphilis group is 80.7 percent and in the Tuskegee study, 100 percent. The specificity of the test is complete in the sense that no reactions were obtained in the nonsyphilitic, the biologic false positive, or the Tuskegee study control group.

Results obtained by the FTA–200 test on the Tuskegee study syphilis group are of particular interest. The test was extremely low in sensitivity (19.5 percent) compared with the TPI test (91.2 percent) and the FTA-ABS test (100 percent). No explanation for the behavior of the FTA–200 test in this serum category has been obtained. However, others have noted the reduced sensitivity of the FTA test. In 50 patients with untreated syphilis of long duration, Eng, Nielsen, and Wereide (*7*) found only 54 percent reactive in the FTA test, while 94 percent were reactive in the TPI test. Wilkinson (*8*) also determined that the FTA–200 test is less sensitive than the TPI test on serums from patients with latent treponemal disease. Wilkinson's results suggested that the sensitivity of the FTA test might be improved without compromising specificity if lower serum dilutions could be used. The FTA-ABS test as described has apparently accomplished this objective. Sensitivity was increased from 19.5 percent to 100.0 percent reactivity in the Tuskegee study without loss of specificity.

Sensitivity and specificity of FTA-ABS procedure in comparison with other tests

Tests	Serum categories and percent reactive				
	SERA study		Biologic false positive (N=38)	Tuskegee study	
	Primary syphilis (N=76)	Normal (N=74)		Syphilis (N=46)	Control (N=36)
FTA–200	36.8	0	0	19.5	0
FTA 1–5 (saline)	100.0	19.7	31.6	100.0	33.3
FTA-ABS	80.7	0	0	100.0	0
TPI	36.8	0	0	91.2	0

The FTA-ABS test as performed in the present study varies from the FTA–200 technique (*6*) in the following ways:

1. Whole serum, rather than diluted, is heated according to the directions given for the standard procedure.

2. A 1:5 dilution of test serum is prepared using Reiter treponeme sonicate as a diluent. The sonicate contains no intact treponemes and is standardized as described.

3. Serum controls consist of a known nonsyphilitic serum demonstrating a high degree of nonspecific reactivity in the FTA 1–5 procedure (without absorption) and a known syphilis serum of low titer. For control of the absorption effect, all serums are diluted 1:5 in buffered saline and compared with the 1–5 absorbed tests. As in the FTA–200, an intensity control for reading the level of reactivity and a nonspecific staining control of antigen and labeled globulin are included.

4. A rotating machine is not used in the FTA-ABS test, but time and temperature of incubation remain as described for the standard procedure.

5. The intensity of test reactions like the FTA–200 test is based on a comparison with a known syphilis serum demonstrating 2+ reactivity at a predetermined dilution. However, with the increased specificity evidenced by the FTA-ABS test, it is believed that 1+ reactions should be considered significant. The control pattern of reading and reporting is as follows:

Reading	*Report*
2+ or stronger fluorescence	Reactive (R)
1+, weakly fluorescent	Weakly reactive (W)
— to ±, barely visible	Nonreactive (N).

Summary

An absorption technique designed to remove nonspecific treponemal antibodies from human serums has resulted in an improved FTA test modification designated as the FTA-ABS test procedure. The sorbing agent is a sonicate of the Reiter treponeme containing common or group nonspecific treponemal antigen. Experimental test results indicate that the new procedure is more than twice as sensitive as the FTA–200 test, and specificity equals that obtainable by the TPI test procedure.

REFERENCES

(*1*) Deacon, W. E., Freeman, E. M., and Harris, A.: Fluorescent treponemal antibody test. Modification based on quantitation (FTA–200). Proc Soc Exp Biol Med 103: 827–829 (1960).

(*2*) Deacon, W. E. and Hunter, E. F.: Treponemal antigens as related to identification and syphilis serology. Proc Soc Exp Biol Med 110: 352–356 (1962).

(*3*) U.S. Public Health Service: Serology evaluation and research assembly (SERA) study, 1956–57. PHS Publication No. 650. U.S. Government Printing Office, Washington, D.C., 1959.

(*4*) Olansky, S., et al.: Untreated syphilis in the male Negro. X. Twenty years of clinical observation of untreated syphilitic and presumably nonsyphilitic groups. J Chronic Dis 4: 177–185 (1956).

(*5*) Olansky, S., Harris, Ad, Cutler, J. C., and Price, E. V.: Untreated syphilis in the male Negro. Twenty-two years of serologic observation in a selected syphilis study group. AMA Arch Derm 73: 516–522 (1956).

(*6*) U.S. Public Health Service: Laboratory procedures for modern syphilis serology. PHS Publication No. 988, revised. U.S. Government Printing Office, Washington, D.C., 1962, pp. 41–46.

(*7*) Eng, J., Nielsen, H. A., and Wereide, K.: A comparative study of fluorescent treponemal antibody (FTA) and treponema pallidum immobilization (TPI) testing in 50 untreated syphilitic patients. World Health Organization Document WHO/VDT/314, WHO/VDT/RES/29, March 1963.

(*8*) Wilkinson, A. E.: The fluorescent treponemal antibody test in the serological diagnosis of syphilis. Proc Roy Soc Med 56: 478–481, June 1963.