Fluorescent Treponemal Antibody Test. Modification Based on Quantitation (FTA-200) (25685)

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The Fluorescent Treponemal Antibody (FTA) test described previously(1) was critically appraised through participation in the Serology Evaluation and Research Assembly (SERA) Study conducted in 1956-57 (2), and shown to produce highly sensitive and specific results. However, since completion of the SERA Study, a new chemical compound, fluorescein isothiocyanate, has been introduced(3) and is employed to produce labeled antiserums of increased potency(4). Since fluorescein labeled antihuman globulin is the treponemal antibody detector system of the FTA test, an improvement in this reagent is reflected in an apparent increase in test reactivity. This means that although the original FTA test had a desirable level of reactivity, the procedure as performed(5) with presently available reagents has not been so measured. In this study, quantitation of the FTA test was undertaken to determine the present sensitivity and specificity of the test as related to new and improved reagents and to ascertain any changes in qualitative procedure which might be employed to obtain a more satisfactory result.

Materials and methods. Test serums were obtained from the Serum Bank, USPHS Serologic Evaluation Study, and TPI Testing Service, all Venereal Disease Research Lab. Serums from the Serum Bank and TPI Testing service had been preserved by freezing at −20°C. SERA Study serums were selected for testing on basis of categories and availability. Qualitative FTA tests were performed by employing a test serum dilution of 1:5 as described earlier(5) and at dilution of 1:200 (FTA-200). Test serums demonstrating Reactive (R) or Weakly Reactive (WR) results at the 1:5 dilution were retested by preparing serial dilutions and determining a 2+ Reactive endpoint. Results obtained by the experimental qualitative procedure (FTA-200) are expressed in terms of Nonreactive (N) or Reactive (R). Weakly Reactive (WR 1+) findings as designated for the FTA test are recorded as Nonreactive (N) in the FTA-200 procedure. Fluorescein labeled horse anti-human-globulin* was used as the treponemal antibody indicator in all tests. The conjugate was diluted at 1:80 (optimal dilution for this particular lot) in phosphate buffered saline, pH 7.2 containing 2% Tween 80. Lyophilized *Treponema pallidum* antigen was employed for smear preparations. Two smears were prepared for each slide/test serum dilution. Of the 2 smears, the most satisfactory in terms of microscopic appearance (numbers and distribution of *T. pallida*) was used for reading and determining final reactions.

Results. Table I illustrates qualitative results obtained by the FTA-200 test based on selection of a critical dilution factor for test performance. These are compared with results obtained by a group of selected tests performed at Venereal Disease Research Lab and previously reported in the SERA Study(2).

In the Presumed Normal Category, 4 serums demonstrated 1+ readings in the FTA-200 test. Reactive(R) titers of these specimens were shown to be 100 dils.† Other test serums had Reactive (R) titers of less than 100 dils. As previously noted, WR (1+) reactions in the FTA-200 test are considered insignificant and were therefore reported as Nonreactive (N). On this basis, almost complete agreement of FTA-200 test results and the comparative test procedures is obtained.

In Early Syphilis, Untreated, consisting of primary and secondary syphilis, quantitative findings ranged from less than 100 dils (primary group) to 25,600 dils in the secondary category. Lack of agreement by various comparative tests is particularly noticeable in the primary group.

In Early Syphilis, Treated, findings appear

* Sylvana Chemical Co. Lot No. 201.

† Dils = Dilution of serum tested; *e.g.*, 100 dils = 1:100 dilution.

TABLE I. Comparison of FTA-200 Test and Other Serologic Tests for Syphilis from Selected Clinical Categories of SERA Study.

	FTA quantitation					
	Range	Mode	FTA-200	TPI	Reiter treponeme	VDRL slide
Presumed normals	<5-100	<5	25-N	1-WR; 24-N	25-N	25-N
Primary syphilis, untreated	<100-3200	200	8-R; 2-N	7-R; 3-N	7-R; 2-WR; 1-N	9-R; 1-N
Secondary syphilis, untreated	800-25,600	6400	10-R	9-R; 1-WR	9-R; 1-WR	10-R
Primary syphilis, treated	200-1600	400	4-R	2-R; 2-WR	3-R; 1-N	4-N
Secondary syphilis, treated	800-3200	1600	5-R	4-R; 1-WR	5-R	3-R; 2-N
Late syphilis, treated	200-25,600	1600	28-R	25-R; 3-WR	25-R; 1-WR; 2-N	17-R; 4-WR; 7-N

to be similar to untreated group but with a trend toward lower titers. Again, agreement of comparative tests appears to be somewhat related to FTA titers; *i.e.*, where titers are high agreements are most satisfactory, but when FTA titers are low, comparative tests tend to obtain a variety of results.

In Table II reproducibility and relative reactivity levels of the qualitative FTA (1:5) and FTA-200 tests are compared. Test specimens consisted of aliquots of 20 serums distributed in the USPHS Serologic Evaluation Study. These were sent to 2 separate sections of Venereal Disease Research Lab for testing. Tests were performed by both sections on 40 code-numbered specimens that were 20 serums in duplicate. Results obtained vividly demonstrate the difference in reactivity levels of the 2 procedures. Of most importance is the reproducibility obtained by both sections using the FTA-200 technic on duplicate serums. This is particularly evidenced by agreement of results obtained on the same serum when tested in the 2 sections.

Discussion. Our findings support the view that the FTA test as described in the 1959 Manual of Serologic tests for Syphilis(5) has become considerably more sensitive than the

TABLE II. Reproducibility of FTA-200 by 2 Laboratories on USPHS Serologic Evaluation Study Specimens for August and September 1959.

Reactive pool serum dilutions	Aug. 1959				Sept. 1959			
	Lab 1		Lab 2		Lab 1		Lab 2	
	FTA-200	FTA 1:5	FTA-200	FTA 1:5	FTA-200	FTA 1:5	FTA-200	FTA 1:5
	R	R	R	R	R	R	R	R
1:2.5	R	R	R	R	R	R	R	R
	R	R	R	R	R	R	R	R
1:5	R	R	R	R	R	R	R	R
	R	R	R	R	R	R	R	R
1:10	R	R	R	R	R	R	R	R
	R	R	R	R	R	R	R	R
1:20	R	R	R	R	R	R	R	R
	N	R	N	R	N	R	N	R
1:40	N	R	N	R	N	R	N	R
	N	R	N	R	N	R	N	R
1:80	N	R	N	R	N	R	N	WR
	N	R	N	R	N	R	N	R
1:160	N	R	N	R	N	R	N	R
	N	R	N	R	N	R	N	R
1:320	N	R	N	WR	N	WR	N	WR
	N	R	N	R	N	N	N	"
1:640	N	R	N	WR	N	WR	N	"
	N	N	N	N	N	R	N	"
N	N	N	N	N	N	N	N	"

similar procedure performed in the SERA Study of 1956-57(2). This increase in sensitivity is particularly evidenced by the frequent occurrence of Weakly Reactive (WR) results obtained in the Presumed Normal category of patients (Table I).

Of various factors which have probably played an important part in increased sensitivity of the FTA test, one must certainly consider the introduction and general use of fluorescein isothiocyanate. Other factors may include use of conjugates prepared from higher titered antiserums and incorporation of Tween 80 to enhance antigen-antibody coupling(5). However, regardless of the actual cause of increased sensitivity of the FTA test, it is obvious that this tends to lower the specificity of procedure in terms of syphilis and seriously affects reproducibility of results.

Our quantitative FTA test yielded high titers in secondary and late syphilis. These range from 200 to 25,600 dils. Primary syphilis, on the other hand, demonstrated low titers ranging from 0‡ to 3200 dils. As has been noted, some specimens in this group produced a 1+ reaction as did some serums obtained from nonsyphilitic donors. If one discounts a 1+ (WR) reaction and reports such reading as Nonreactive (N), as is done in the FTA-200 test, false positive reports are avoided without appreciable loss of ability to detect syphilitic patients. Studies to be reported later indicate that the low grade fluorescence obtained when testing presumably nonsyphilitic patients (1:50 and 1:100) is probably due to treponemal antibodies not associated with syphilis. It should be noted also that in no instance was strong fluorescence or a 2+ Reactive (R) result obtained in the Presumed Normal group at the critical 1:200 dilution.

Summary. 1. A modified FTA test technic designated as FTA-200 is described and recommended on the basis of results obtained by examining well-documented serum specimens from various syphilis categories and a Presumed Normal group. 2. The FTA-200 test possesses a greater degree of reproducibility than the FTA test when results were compared on duplicate serums.

‡ 0 dils = less than 200 dils in the FTA-200 test.

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Plasma Clearance and Glucuronide Conjugation of 11-Desoxycortisol (Substance S) in Man.* (25686)

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The metabolic fate of a number of adrenocortical steroids in the peripheral circulation of man has been described by Peterson(1). Recently Touchstone *et al.*(2) reported that *11-desoxycortisol* was secreted by the human adrenal in significant quantities, amounting to approximately one-tenth the concentration of *cortisol* in adrenal venous blood. Rate of removal of 11-desoxycortisol from plasma and rate of appearance of its glucuronide-conjugated metabolites in "normal" subjects and in patients with liver disease and hypothyroidism are reported here.

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