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THE FTA-ABS TEST FOR SYPHILIS*†

PERFORMANCE IN 1,033 PATIENTS

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During the 20th century, screening tests using nontreponemal lipoidal antigens have evolved from the old Wassermann test to such excellent ones as the VDRL and Kahn flocculation tests and the Kolmer complement-fixation test. These tests are easily performed in small laboratories and are almost invariably reactive in patients who have any form of active syphilis other than primary syphilis. Although the cardiolipoidal antigen tests are sensitive, they are not specific for syphilis, and other clinical or laboratory confirmation is therefore needed.

The TPI test has been the standard confirmatory serological test since the early 1950s. Its sensitivity has been proved repeatedly. Its specificity also is excellent because the antigen used in the test is the aetiological agent of syphilis. However, the technical difficulty and expense of TPI testing has limited its use to a very few specialized laboratories, and has prompted a search for other confirmatory tests that are also highly specific and sensitive but more easily performed.

In the search, fluorescent antibody techniques have been the most rewarding. Coons, Creech, and Jones (1941) first described the technique of conjugating antibody molecules with fluorescein. Despite conjugation with fluorescein, an antibody will retain its capacity to react specifically with its antigen. There are both direct and indirect fluorescent antibody techniques. Direct techniques use antibodies labelled with fluorescein as a histochemical stain for identifying their specific antigens, or, conversely, the antigens are used to identify their antibodies (Thomason, Cherry, and Moody, 1957; Coons and Kaplan, 1950).

Indirect techniques (which are more suited to serological testing) apply unidentified, unlabelled antibodies to known antigens or *vice versa* (Coons, 1951; Harris, Deacon, and Smith, 1957). After the antibodies have had sufficient time to combine with the antigen, a washing procedure removes all but the attached antibodies. The next step is the addition of fluorescein-labelled antiglobulin which will attach to antibody globulin. Fluorescence proves that an antigen-antibody reaction has taken place. The antibody is therefore identified indirectly.

The principle that Coons, and others (1941) described has been successfully applied to many facets of microbiology and immunology. The outcome of its application to serological testing for syphilis has been the development at the Venereal Disease Research Laboratory of a test that rivals the TPI for both specificity and sensitivity. Dr Deacon, who is now director of that laboratory, and his co-workers developed the original fluorescent treponemal antibody test, an indirect technique (Deacon and Hunter, 1962; Deacon, Falcone, and Harris, 1957). Its major steps are as follows:

(1) *T. pallidum* organisms the specific antigen, are fixed on slides.

(2) The patient's serum is applied to the organism to allow combination of antibodies (located in the globulin fraction of the serum) with the treponemes.

(3) Species specific, fluorescein-conjugated antibodies (antihuman globulin produced in goats) are then applied to the slide.

Any antibodies from the patient's serum that combined with the treponemes in Step 2 now react

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with the fluorescein-labelled antiglobulin produced in goats. The combined products can be identified as fluorescent treponemes when viewed under an ultraviolet light microscope.

When this technique was used to detect the presence of antibodies in early syphilis (Montgomery, Suhrland, and Knox, 1960), they were usually detected only in low dilutions in subjects with primary syphilis and in high dilutions in subjects with secondary syphilis. In subjects with latent syphilis, the titres again dropped to low levels.

Although the original FTA test appeared promising, it soon became evident that further improvement was needed. It was useful to test sera in low dilutions (1:5) in order to detect latent syphilis sensitively. However, this dilution produced non-specific positive reactions in 20 per cent. of a group of patients who did not have syphilis. Therefore, the test was set at 1:200 as the dilution that gave the best reproducibility and balance between sensitivity and specificity (Deacon, Freeman, and Harris, 1960).

Further study suggested that a major cause of the test's non-specificity was the occurrence of common or group specific antigens shared by both *T. pallidum* and saprophytic treponemes. Non-specific reactions previously encountered at the 1:5 dilution were eliminated by absorbing non-specifically reacting sera with ultrasonically disintegrated Reiter treponemes (Reiter sonicate) of the non-pathogenic Reiter spirochaete (Hunter, Deacon, and Meyer, 1964). Absorption with the Reiter material has now been incorporated into the testing of all sera and the improved test is designated the FTA-ABS test.

In co-operation with Deacon's group at the Venereal Disease Research Laboratories, the Houston City Laboratory and Social Hygiene Clinic recently evaluated the FTA-ABS test. The following account includes results from the first 1,033 subjects of the study which is still in progress.

Method and Material

Most of the subjects were patients who came to the Clinic for diagnosis and treatment. Patients with symptoms usually came either spontaneously or in response to epidemiological investigations. Most asymptomatic patients came for evaluation of serological reactions found at the city-county hospital or other laboratories.

Thorough physical examinations were carried out on each patient and histories were taken. For maximum accuracy, examinations were repeated if the diagnosis was not obvious. Serum was obtained from each subject for VDRL and FTA-ABS tests which were performed by the Houston Laboratory. A portion of each serum was sent to the Venereal Disease Research Laboratory, where VDRL and TPI tests were performed on all specimens. The FTA-ABS test was repeated whenever the results of the Houston FTA-ABS and the TPI tests did not agree.

The study group included 37 patients with primary syphilis, 41 with secondary syphilis, 18 with late syphilis, and 22 with congenital syphilis; sixty other patients related a history of syphilis treated in the past with medications considered adequate at the time of treatment.

There were 554 "problem" cases which did not belong to any of the previously mentioned categories. Although their VDRL tests were positive, neither their histories nor their physical findings supported a diagnosis of syphilis, and in most of them the possibility of neurosyphilis was eliminated by cerebrospinal fluid examinations. In patients such as these the choice of diagnosis between late latent syphilis or a biological false positive reaction depends on the results of specific treponemal tests.

In addition to the patients described above, the same tests were carried out on sera from 301 control subjects in whom the VDRL slide tests and TPI tests were non-reactive and who had no symptoms or history of syphilis.

Criteria of Classification

To formulate a precise diagnosis in syphilis, one must combine clinical findings, laboratory studies, and accurate historical information. This is difficult in a disease with such varied manifestations. Rigid diagnostic criteria were used at certain stages. For example, patients thought to have primary syphilis were not included in the study unless the diagnosis was verified by a positive dark-field examination. A diagnosis of secondary syphilis was allowed without a positive dark-field result only when classical skin lesions accompanied a VDRL titre of 1:8 or greater, most, however, were dark-field positive. The diagnosis of neurosyphilis was well defined because a positive VDRL slide test in the cerebrospinal fluid was a prerequisite.

The remaining categories required more individual evaluation. Here diagnostic precision depended more on the ability and thoroughness of the examining clinician. Congenital syphilis was securely diagnosed if the stigmata of this process were present in a patient with a positive VDRL test. However, congenital syphilis was considered the most likely diagnosis in TPI-reactive patients whose serological reactions were first observed during prepubertal years and in TPI-negative patients previously treated for congenital syphilis.

The category of previously-treated latent syphilis comprised sixty patients with a credible history of treatment for syphilis. Some were uncertain of the stage of disease at the time of treatment but did not recall having lesions. All were diagnosed as having been treated for latent syphilis regardless of the findings in the TPI tests. This is because the TPI test may become negative after treatment for syphilis.

The most difficult differential diagnosis in syphilis is that between untreated latent syphilis and biological false reactions to the VDRL test. For the purposes of this report we considered patients in whom this choice remained as "problem" cases.

The control group were those whose history and physical examinations revealed no evidence of syphilis and in whom the VDRL and TPI tests were negative. Most of them were army inductees, and the group represented the entire socio-economic spectrum in the area. However, it can never be certain that all members of any group have never been infected with syphilis. For this reason, screening by means of the VDRL and TPI tests was used to obtain a better control group.

Despite occasional difficulties in classifying patients, each subject in this series received as complete an evaluation as possible. Laboratory examinations, physical examinations, and histories were repeated when the diagnosis was not completely apparent. We believe that the possibility of a few errors in diagnosis does not prevent a realistic interpretation of the results.

Results

Primary Syphilis (Table I).—The FTA-ABS became reactive at approximately the same time as the VDRL. Among 37 patients with dark-field positive primary lesions, the test was reactive in 32 (87 per cent.), and the VDRL in 29 (80 per cent.). Sera from 26 patients reacted to both tests, six to the FTA-ABS only, and three to the VDRL slide test only. The TPI was reactive in 21 cases (57 per cent.). No patient with primary syphilis reacted to the TPI test alone.

Secondary Syphilis (Table I).—The VDRL and other lipoidal antigen tests are said to be reactive in all patients with secondary syphilis. Such was the case in this study. The FTA-ABS performed admirably, showing reactivity in all 41 patients with secondary syphilis. The TPI was also positive in all patients.

Late Syphilis (Table I).—in eighteen patients with late syphilis (fifteen neurosyphilis and three cardiovascular syphilis) the FTA-ABS and TPI were in 100 per cent. agreement. All eighteen patients reacted to both tests.

Congenital Syphilis (Table I).—All 22 patients with congenital syphilis gave positive reactions to the FTA-ABS test and 21 to the TPI test. One patient who reacted to the FTA only had been treated in the past.

Previously-Treated Syphilis (Table I).—The two tests were in good agreement in the sixty patients previously treated for latent syphilis; 48 were reactive to both tests, seven to the FTA-ABS only, and two to the TPI test only. Three patients were non-reactive to both tests.

Problem Cases (Table I and Table II, opposite).—Of the remaining 554 patients with positive VDRL tests and no clinical evidence on which to base the diagnosis, the TPI was reactive in 453 and the FTA-ABS in 486, the latter being positive in 451 of the TPI-positive cases. Therefore, in only two cases was the TPI positive and the FTA-ABS negative. In 35 of the problem cases the FTA-ABS was positive, but the TPI negative, in 66 neither test was positive. The last group are considered to be biological false positive reactors.

Control Subjects (Table I).—None of the 301 control subjects who had negative TPI and VDRL slide tests reacted to the FTA-ABS test.

TABLE I

REACTIVITY OF VDRL, TPI, AND FTA-ABS TESTS ON SERA FROM 1,033 CLINICALLY DEFINED DONORS

Clinical Category		No. of Patients	Positive Tests					
			VDRL Slide		TPI		FTA-ABS	
			No.	Per cent.	No.	Per cent.	No.	Per cent.
Syphilis	Primary	37	29	80	21	57	32	87
	Secondary	41	41	100	41	100	41	100
	Late	18	18	100	18	100	18	100
	Congenital	22	22	100	21	95	22	100
	Previously Treated	60	60	100	50	83	55	91
Problem		554	554	100	453	82	486	88
Control*		301	0	0	0	0	0	0

* Control sera were obtained from Army inductees with non-reactive VDRL slide and TPI tests.

TABLE II

FTA AND TPI RESULTS IN 554 DIAGNOSTIC PROBLEM CASES

Test	Result	Patients	
		No.	Per cent.
FTA-ABS .. TPI	+ +	451	81
FTA-ABS .. TPI	− −	66	12
FTA-ABS .. TPI	+ −	35	6·3
FTA-ABS .. TPI	− +	2	0·36
Total		554	100

Discussion

A good serological test should be both specific and sensitive. These two terms are used according to the following definition:

"*Sensitivity* is defined as the percentage of reactive or weakly-reactive results obtained among specimens from syphilitic donors; *specificity* as the percentage of negative results obtained among specimens from non-syphilitic donors" (U.S. Communicable Disease Center, 1956-57). The FTA-ABS appears to have a high degree of specificity. This was demonstrated by its non-reactivity among control subjects.

It is not disturbing that among patients previously treated for syphilis, some reacted to the TPI test and not to the FTA-ABS test and *vice versa*. This discrepancy is expected because, after treatment, antibody patterns vary with the individual host and with every test procedure. Total agreement between two tests for syphilis is impossible unless both detect identical antibodies with equal sensitivity. The close agreement of the two tests here investigated indicates that each measures an antibody present for a long period after treatment, although either test may be the first to become negative after treatment.

Although specific tests are neither necessary nor desirable for the diagnosis of early syphilis, early reactivity lessens the possibility of making a wrong diagnosis in certain clinical situations. For example, it may be difficult to make a correct diagnosis if the patient's primary lesion is undetected or if he is first seen between the primary and secondary stages of syphilis. At that time he may fulfil the criteria (a reactive serology without clinical signs of activity) if not the concept of latent syphilis. If such a patient were given a specific test that lacked sensitivity, he might be incorrectly labelled as a false reactor. The TPI would occasionally fail in these cases while the FTA would usually reveal them.

Although secondary syphilis, congenital syphilis with stigmata, or late manifest syphilis also should be diagnosed without resorting to specific treponemal tests, it is gratifying that the FTA-ABS reactivity is extremely high in these patients.

The only important discrepancies found between the two tests in this study occurred in patients whom we call "diagnostic problem cases". Such patients give no history of syphilis, have no clinical findings to support the diagnosis, and have a reactive reagin test. In the past, the final diagnosis was based on results of a TPI test. There is good evidence to justify this arbitrary approach, for the TPI has been a reliable test. The incidence of biological false positive reactions to it is low, both among normal subjects and among patients with diseases that produce false positive reactions with lipoidal antigens. Also the incidence of TPI reactors in latent syphilis approaches 100 per cent. and does not tend to diminish with time (Olansky, Harris, Cutler, and Price, 1956).

Of the 101 problem cases with negative TPI tests in this series, 35 reacted to the FTA-ABS test. Considering the performance of the test in other patients, we do not believe all or even many of the 35 were in fact false positive reactors. Several facts support our opinion. In the group of patients known to have been previously treated for syphilis, more reacted to the FTA-ABS than to the TPI test. Also, the study subjects were from a low socio-economic level and were poor historians in general. Some may have been treated with antibiotics for other infections, and others may have forgotten treatment actually given for syphilis. Consequently, there is a good possibility that many of them had had previous treatment that was not elicited.

These data concerning the FTA-ABS are impressive. Although further evaluation is needed, the indications are that it will eventually prove to be the best specific treponemal test. It has advantages over other specific tests in general use. The relation of the FTA-200 to the FTA-ABS has been previously discussed and the advantages of the latter seem firmly established. Its sensitivity is undoubtedly greater than the Reiter protein complement-fixation test, which does not approach the sensitivity of the TPI test. The antigen used in the RPCF is essentially that used to absorb non-specific antibodies from serum in the FTA-ABS. This fact alone should make the RPCF obsolete when the FTA-ABS becomes available.

The TPI, although an outstanding test, is expensive and cumbersome. Its other disadvantages include possible invalid results due to antibiotics taken by patients before the sera are obtained and

bacterial contamination or toxic effects from rubber stoppers used in specimen containers.

Evaluation of the FTA-ABS test is being continued. Several areas need investigation. Its performance may prove to be inferior in patients with diseases known to give false reactions to other serological tests. Possibly the dysglobulinaemias have abnormal proteins which could combine with *T. pallidum* and produce false reactions. One group of investigators has suggested that patients with diseases in which antinuclear antibodies are present may be false reactors to FTA tests (Neblett, Merriam, Burnham, and Fine, 1964). These possibilities will be investigated. However, such problems probably could be overcome with absorption techniques.

Another possibility is that technical problems may prevent the production of large quantities of standardized Reiter sonicate. This material is used to absorb non-specific antibodies which cross-react with *T. pallidum*. The absorbent must be inexpensive, readily available, reproducible and obtainable in sufficient quantity for widespread usage.

Summary

Absorbing sera with disrupted Reiter treponemes eliminates most false positive reactions encountered previously in the fluorescent treponemal antibody test for syphilis. The improved test, the FTA-ABS, was studied in 1,033 patients and was found to be as sensitive as or more sensitive than the TPI test in all stages of syphilis. In patients with primary syphilis the test was reactive more often than the TPI and as often as the VDRL test. In patients previously treated for syphilis, it also was reactive more often than the TPI test. In other stages the FTA-ABS was reactive as often as the TPI test.

The specificity of the FTA-ABS test has not been completely evaluated. Although there were no reactions among normal controls, the study of its performance among patients with diseases known to produce false positive serological reactions has not been completed.

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Le test FTA-ABS pour la syphilis en 1,033 malades

RÉSUMÉ

Le sérum qui a absorbé les tréponèmes fragmentés de Reiter élimine les réactions positives fausses rencontrées précédemment en faisant le test fluorescent des anticorps des tréponèmes pour la syphilis. Ce test amélioré, étudié chez 1,033 malades, s'est montré plus sensible que le test TPI (réaction de l'immobilisation du tréponème) dans tous les stages de la syphilis. Chez les malades souffrant de syphilis précoce ce test a donné une réaction positive plus souvent que le TPI et aussi souvent que le VDRL. Chez les syphilitiques traités, il s'est aussi montré plus souvent réactif que le TPI. Dans les autres stages ce test s'est révélé réactif aussi souvent que le test TPI.

La spécificité de ce test n'a pas été encore complétement évalué. Bien que chez les contrôles normaux il n'y ait pas eu de réactions, l'étude de sa valeur chez les malades atteints de maladies connues pour la production des réactions positives fausses n'a pas été términée.