

ABSORBED FLUORESCENT TREPONEMAL ANTIBODY (FTA-ABS) TEST*

COMPARISON WITH THE FTA-200 AND TPI TESTS ON 1,056 PROBLEM SERA

BY

N. A. JOHNSTON AND A. E. WILKINSON

Venereal Diseases Reference Laboratory (PHLS), The London Hospital, London, E.1

Deacon and Hunter (1962) have shown that the fluorescent treponemal antibody (FTA) test detects two separate antibodies in syphilitic sera. One, a group antibody, reacts with other treponemes, such as the Reiter organism, *Treponema microdentium*, and *Treponema zuelzeriae*, as well as with *Treponema pallidum*, presumably because of an antigen shared in common by these organisms. Removal of group antibody from syphilitic sera by absorption with Reiter treponemes allows the detection of a second antibody which is thought to be specific for *T. pallidum*. Group antibody is present to low titres in the majority of normal, non-syphilitic sera, possibly being produced in response to the presence of commensal treponemes in the body. Wilkinson and Rayner (1966) have shown that it also occurs in the course of syphilitic infection and that, except in secondary syphilis, it appears to preponderate over the antibody specific for *T. pallidum*. This has also been found to occur in infections with yaws (Wilkinson, unpublished data).

In the FTA-200 test described by Deacon, Freeman, and Harris (1960), sera are tested at a dilution of 1 in 200 which is above the threshold of group antibody found in the great majority of non-syphilitic sera. Despite the sensitivity of the indirect fluorescence technique, this dilution reduces the potential sensitivity of the method and various means have been advocated for the removal of group antibody from sera so that tests can be made for the presence of the antibody specific for *T. pallidum* alone. This has been done by absorption of sera with intact Reiter treponemes, by a sonicate of these organisms or by a heated filtrate of a culture of Reiter treponemes (Hunter, Deacon, and

Meyer, 1964; Hunter, 1964; Betz, 1966; Deacon, Lucas, and Price, 1966; Knox, Short, Wende, and Glicksman, 1966). The use of intact or ultrasonically disintegrated Reiter treponemes presents technical difficulties, but the heated culture filtrate can be prepared in sufficient quantity and its reactivity standardized and so was used in the present investigation.

Material and Methods

Sera

Tests were made on all sera sent to the laboratory for TPI tests during a period of 4 months. These were "problem" sera, usually referred because tests for reagin had been found positive in other laboratories. FTA-200, Reiter protein complement-fixation (RPCF), cardiolipin Wassermann reaction (CWR), and VDRL slide tests were also performed. During the period reviewed there were 1,056 sera on which valid results had been obtained by all six tests.

FTA-ABS Test

This was performed according to the technique recommended by the Venereal Diseases Research Laboratory, Communicable Disease Center, Atlanta, Ga. (Report, 1968). The fluorescein-conjugated anti-human gamma globulin serum and the sorbate were prepared in the laboratory. The latter had a working titre of 1 in 3; at this dilution it completely abolished the reactivity of a non-specific control serum which gave +++ fluorescence at a dilution of 1 in 25 in saline but did not significantly reduce the fluorescence given by a known positive serum. The tests were read on a Reichert Zetopan microscope using a 2 mm. BG 12 exciter filter and appropriate barrier filters. Sera giving definite ++ to ++++ fluorescence were accepted as reactive. Tests were repeated on sera giving borderline readings between + and ++, if these were confirmed, the results were also classed as reactive.

* Received for publication May 10, 1968.

TABLE I
COMPARISON OF THE RESULTS OF THE TPI TEST WITH THE FTA-ABS AND
FTA-200 TESTS ON 1,056 "PROBLEM" SERA.

TPI	Positive 563				Doubtful 59				Negative 434			
	Positive 443		Negative 120		Positive 39		Negative 20		Positive 83		Negative 351	
FTA-200	Pos. 441	Neg. 2	Pos. 107	Neg. 13	Pos. 37	Neg. 2	Pos. 14	Neg. 6	Pos. 30	Neg. 53	Pos. 25	Neg. 326

Results

A comparison of the results of the TPI test with those of the two fluorescence tests is given in Table I.

If the TPI results are taken as the standard for comparison, positive and doubtful results being grouped together as reactive, there was overall agreement between the TPI and FTA-200 in 78.9 per cent. of the sera and between the TPI and the FTA-ABS test in 92.7 per cent. 622 sera were reactive in the TPI test; of these, 77.5 per cent. were reactive in the FTA-200 and 96.4 per cent. in the FTA-ABS test. These results show clearly that the FTA-ABS test is considerably more sensitive than the FTA-200; even in the group of 59 sera which gave doubtful TPI results, the FTA-ABS was found reactive in 51 (86.5 per cent.) but the FTA-200 gave only 39 (66 per cent.) positive results.

In the group of 434 sera which gave negative TPI results, the FTA-200 was read as reactive in 83 instances, many of these being borderline reactions. The FTA-ABS was also found reactive in thirty of these and with a further 25 sera on which the FTA-200 was negative. Examination of the clinical information—often very scanty—supplied with these sera gave the results summarized in Table II.

TABLE II
CLINICAL INFORMATION AVAILABLE ABOUT
PATIENTS WHOSE SERA GAVE NEGATIVE TPI BUT
POSITIVE FLUORESCENCE TESTS

Group	No. of Cases	FTA-200+ FTA-ABS+	FTA-200- FTA-ABS+	FTA-200+ FTA-ABS-
History of Syphilis or Yaws Lesions possibly Syphilitic or Diagnosis ? Syphilis	19	8	10	1
No information	24	8	3	13
	65	14	12	39

From these data it appears that in a proportion of these patients there was clinical evidence to

substantiate the reactive fluorescence tests. Nineteen of these gave a definite history of previous syphilis or yaws; the FTA-200 test was positive in nine but the FTA-ABS test was positive in all but one.

The TPI test has found its most useful application in the differentiation of clinically latent treponemal disease from biological false positive reactions with tests for reagin. Although a positive TPI test is good evidence of treponemal disease, past or present, failure to demonstrate immobilizing antibody does not exclude such infection with the same degree of certainty because the antibody appears rather late in the primary stage and may be absent in a minority of long-standing cases. Tuffanelli, Wuepper, Bradford, and Wood (1967) re-examined 100 patients who had been presumed to be biological false positive reactors on the basis of one or more negative TPI tests. Fifty of these had negative TPI and FTA-ABS tests; in 38, although the TPI was again found negative, the FTA-ABS test was positive and in seven of these patients clinical evidence of late syphilis was found. The remaining twelve patients were found to have positive TPI and FTA-ABS tests and six showed clinical evidence of late syphilis. The authors consider that the FTA-ABS test should be used to identify chronic BFP reactors because of its high sensitivity. Lassus, Mustakallio, Aho, and Putkonen (1967) have also re-examined 57 patients classed as chronic BFP reactors because of negative TPI tests. They found that sera from six of these gave weakly reactive and one a reactive FTA-ABS test; they consider that these seven patients should not be regarded as definite BFP reactors because of these findings.

In the present series there were 171 sera in which the TPI test was negative but one or more of the tests for reagin positive, and a further seventeen on which the Reiter protein complement-fixation test was also positive. On purely serological grounds, therefore, these reactions might be suspected of being falsely positive. The FTA-ABS test was found positive in thirteen of the former group and in eight of the latter. The available information

TABLE IIIA
CLINICAL INFORMATION AVAILABLE ABOUT THIRTEEN PATIENTS WHOSE SERA GAVE NEGATIVE
TPI TESTS BUT POSITIVE FTA-ABS AND REAGIN TESTS

Case No.	Test					Clinical Data
	RPCF	VDRL	CWR	FTA-200	FTA-ABS	
1	-	R4	++	0	++	? chronic BFP reactor
2	-	-	+	++	++	Contact of primary syphilis
3	-	R1	++	++	++	West Indian male. Penile sore 6 wks ago. Contact has vaginal ulcer
4	-	-	+	0	++	Coloured male, treated for ? early syphilis 1965
5	-	R2	++	0	++	Male aged 65. Diabetic
6	-	-	++	0	++	Blood donor
7	-	WR	-	++	+++	Female. Husband had syphilis 40 yrs ago
8	-	R2	++	++	++	Male, penile sores. Various recent exposures
9	-	WR	-	++	++	Male aged 77. Similar results on second serum
10	-	WR	+	++	++	Female, arthritis metacarpophalangeal joints. Similar results and positive TPI (63 per cent. s.i.) on second specimen
11	-	WR	-	0	++	Mental disturbance. Treated for "VD" many years ago
12	-	-	+	0	++	Coloured pregnant female
13	-	R1	++	++	++	Female aged 64. Choroiditis and absent ankle jerks

WR = Weakly reactive.

TABLE IIIB
CLINICAL INFORMATION AVAILABLE ABOUT EIGHT PATIENTS WHOSE SERA GAVE NEGATIVE TPI TESTS
BUT POSITIVE FTA-ABS, REAGIN, AND REITER PROTEIN COMPLEMENT-FIXATION TESTS

Case No.	Test					Clinical Data
	RPCF	VDRL	CWR	FTA-200	FTA-ABS	
1	++	WR	-	++	++	Pregnant. Similar results on second specimen
2	+	R2	++	0	++	"Suspicious" STS in previous pregnancy 8 yrs ago. No treatment given then
3	+	WR	-	0	++	Female West Indian. Pregnant. ? yaws scars on legs
4	+	-	+	++	++	Female aged 60. ? syphilis
5	++	-	±	0	++	Male aged 51. History of treated syphilis in the past
6	±	WR	±	+	++	Male aged 60. Poor arterial blood supply, R. arm
7	±	R1	-	++	++	Darkground positive primary syphilis
8	++	-	++	0	++	Pregnant, no antenatal care. Child clinically normal

WR = Weakly reactive.

about these patients is shown in Tables IIIA and IIIB (above).

In the thirteen patients listed in Table IIIA, a diagnosis of treponemal infection is thought probable in eight (Cases 2, 3, 4, 7, 8, 10, 11, and 13) and in three of the eight patients described in Table IIIB (Cases 3, 5, and 7).

37 specimens of cerebrospinal fluid were examined by the FTA-ABS and TPI tests. Complete agreement was found, nine specimens being positive and 28 negative by both methods.

To assess the performance of the FTA-ABS test in normal individuals presumed to be free from treponemal disease, sera from 107 blood donors were tested. All gave negative results with the RPCFT, CWR, and VDRL tests and the FTA-ABS tests were all found to be negative.

Discussion

The FTA-ABS test is technically no more difficult to perform than the FTA-200 test. The results obtained in this series of problem sera show that the absorption procedure considerably en-

hances the sensitivity of the test without compromising its specificity. Deacon and others (1966), in a survey of 2,252 sera, showed that the FTA-ABS test at least equalled the TPI test in sensitivity in tests on sera from patients with untreated and treated syphilis at all stages. They found it considerably more sensitive than the TPI in untreated and treated primary syphilis. Three sera (0.8 per cent.) from 384 presumed normal patients gave positive FTA-ABS tests at one of the laboratories participating in the study, but these were found to be negative when re-tested in the authors' laboratory. Garner, Grantham, Collins, and Roeder (1968) have compared the FTA-ABS test with the TPI on 841 problem sera and found 95.1 per cent. agreement; discrepancies in which the FTA-ABS test alone was positive were mainly found when testing sera from patients with primary syphilis or with a history of treated syphilis. Harner, Smith, and Israel (1968) reported on the use of the absorbed test in the investigation of 1,985 patients in whom late ocular or neurosyphilis was to be excluded; they found it reactive in 41 per cent., but the TPI

was reactive in only 29 per cent. of the 1,298 sera tested by it. 126 sera gave negative TPI but positive FTA-ABS test results; on clinical grounds these patients were thought to have syphilis. Atwood, Miller, Stout, and Norins (1968) re-examined 67 patients who had been diagnosed and treated for latent or late syphilis 13 years previously on the basis of positive TPI and reagin tests. The TPI test was found to have reversed to negative in seven of these patients, all with late latent syphilis, but the FTA-ABS test was positive in all save one, who was also TPI-negative.

The results obtained in the present study on a very selected group of sera have confirmed the high sensitivity of the absorbed test and suggest that it has an acceptable level of specificity. It has proved of considerable value in the investigation of "problem" sera and it is thought that it should supersede the less sensitive FTA-200 test. Used in conjunction with tests for other antibodies produced in treponemal infection (reagin and RPCF antibody), it should be most helpful in assessing the specificity of reactions in problem sera and materially reduces the numbers of sera on which TPI tests are needed.

As yet there is no published information on the nature of the active principle in the sorbing agent. The heated culture filtrate is a very crude product, and although it works well in practice, further studies with a view to its isolation and purification are desirable. Work by Király, Jobbágy, and Kováts (1967) suggests that absorption of sera by Reiter treponemes may not necessarily remove reactivity with all other cultivable treponemes so that a sorbing agent prepared from a wider range of these organisms might be advantageous.

Summary

- (1) The results of the FTA-ABS test and FTA-200 test are compared with those of the TPI test on 1,056 sera presenting serological problems.
- (2) The FTA-ABS test was found to be considerably more sensitive and to be more specific than the FTA-200 test.
- (3) Complete agreement between the FTA-ABS and TPI tests was found on 37 specimens of cerebrospinal fluid. No positive FTA-ABS test results were found on sera from 107 presumed normal persons.

Our thanks are due to Messrs C. J. Storey and H. Ferguson for their technical assistance and to Lt.-Col. R. C. Stewart, R.A.M.C. College, Millbank, for supplying donor sera.

REFERENCES

- Atwood, W. G., Miller, J. M., Stout, G. W., and Norins, L. C. (1968). *J. Amer. med. Ass.*, **203**, 549.
- Betz, A. (1966). *Bull. Wild Hlth Org.*, **34**, 154.
- Deacon, W. E., Freeman, E. M., and Harris, A. (1960). *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 827.
- and Hunter, E. F. (1962). *Ibid.*, **110**, 352.
- , Lucas, J. B., and Price, E. V. (1966). *J. Amer. med. Ass.*, **198**, 624.
- Garner, M. F., Grantham, N. M., Collins, C. A., and Roeder, P. J. (1968). *Med. J. Aust.*, **1**, 404.
- Harner, R. E., Smith, J. L., and Israel, C. W. (1968). *J. Amer. med. Ass.*, **203**, 545.
- Hunter, E. F. (1964). In "Proceedings of World Forum on Syphilis and Other Treponematoses, Washington, D.C., 1962", p. 269. U.S. Public Health Service Publication No. 997.
- , Deacon, W. E., and Meyer, P. E. (1964). *Publ. Hlth Rep. (Wash.)*, **79**, 410.
- Király, K., Jobbágy, A., and Kováts, L. (1967). *J. invest. Derm.*, **48**, 98.
- Knox, J. M., Short, D. H., Wende, R. D., and Glicksman, J. M. (1966). *Brit. J. vener. Dis.*, **42**, 16.
- Lassus, A., Mustakallio, K. K., Aho, K., and Putkonen, T. (1967). *Acta. path. microbiol. scand.*, **69**, 159.
- Tuffanelli, D. L., Wuepper, K. D., Bradford, L. L., and Wood, R. M. (1967). *New. Engl. J. Med.*, **276**, 258.
- V.D.R.L., Staff Report. (1968). *Hlth Lab. Sci.*, **5**, 23.
- Wilkinson, A. E., and Rayner, C. F. A. (1966). *Brit. J. vener. Dis.*, **42**, 8.

Le test fluorescent de l'anticorps absorbé du tréponème (FTA-ABS) Comparaison au test fluorescent de l'anticorps du tréponème 200 (FTA-200) et au test de l'immobilisation du tréponème (TPI) sur 1,056 sérums présentant des problèmes sérologiques

RÉSUMÉ

- (1) Les résultats du test FTA-ABS et ceux du test FTA-200 sont comparés à ceux du test TPI faits avec 1.056 sérums présentant des problèmes sérologiques.
- (2) Le test FTA-ABS a été trouvé comme étant beaucoup plus sensible et aussi beaucoup plus spécifique que le test FTA-200.
- (3) Une concordance complète entre les tests FTA-ABS et TPI a été trouvé en examinant 37 spécimens du liquide céphalo-rachidien. Aucun résultat positif n'a été rencontré quand 107 sérums obtenus de personnes normales ont été soumis au test FTA-ABS.