Brit. J. vener. Dis. (1967), 43, 186.

FLUORESCENT TREPONEMAL ANTIBODY INHIBITION TEST*

BY

A. E. WILKINSON

Venereal Diseases Reference Laboratory (Public Health Laboratory Service), The London Hospital, London, E.1

The fluorescent treponemal antibody (FTA-200) test has been shown to detect at least two types of antibody in syphilitic serum; one of these, which can be removed by absorption with Reiter treponemes, is thought to be a group antibody which reacts with an antigen shared by *Treponema pallidum* and other treponemes (Deacon and Hunter, 1962). A second antibody, which remains after such an absorption, is thought to be specific for *T. pallidum*. The group antibody is present in many non-syphilitic sera in low titre, possibly being produced in response to the presence of commensal treponemes; it rarely reaches a titre of 1 in 200, the dilution at which sera are tested in the FTA-200 test. Wilkinson and Rayner (1966) have shown that, except in early syphilis, the group antibody preponderates in syphilitic sera. Non-specific reactions may occur in the FTA-200 test apart from the presence of an unusually high titre of group antibody in non-syphilitic sera; Fife (1964) reported such reactions with some sera containing abnormal macroglobulins and Wilkinson and Rayner (1966) demonstrated that sera containing rheumatoid factor could give positive FTA tests if the treponeme suspension used was partially sensitized with antibody from the rabbit from which it was prepared.

Absorption procedures with intact Reiter treponemes, a sonicate of these organisms, or heated culture filtrates (Hunter, Deacon, and Meyer, 1964; Hunter, 1964; Betz, 1966; Deacon, Lucas, and Price, 1966) have been claimed to improve the sensitivity and specificity of the FTA test by removing the group antibody from sera. It has not been shown that these procedures will abolish reactivity of sera containing abnormal globulins in the FTA test. Inhibition of the fluorescence of antigen with fluorescein-labelled antibody by first saturating the antigen receptors with unlabelled antibody of known specificity has been widely used as a check on the specificity of fluorescent antibody reactions. Ruczkowska (1965) has described a test for the presence of anti-treponemal antibodies in sera based on the ability of such sera to block fluorescence of treponemes by conjugated syphilitic serum. The same principle has been used in the present investigation by a slightly modified technique.

* Received for publication May 9, 1967.

Material and Methods

Sera

These were selected from specimens sent to the laboratory for TPI tests and were mainly problem sera, lipoidal antigen tests having been found positive elsewhere and confirmation of the specificity of these results being required. Sera from blood donors which had given negative VDRL slide tests were used as a non-syphilitic control series.

FTA-200 and TPI Tests

The methods described by Wilkinson and Rayner (1966) were used.

Preparation of Conjugated Syphilitic Antibody

Globulins were separated from a pool of sera from four patients with dark-field-positive primary syphilis and four with secondary syphilis by precipitation with 40 per cent. saturated ammonium sulphate. After dialysis, the globulins were conjugated with fluorescein isothiocyanate, using 0·05 mg. FITC per 1·0 mg. globulin (Nairn, 1962). Unreacted FITC was removed by passage through a Sephadex G-25 column and the eluate concentrated back to the original volume of serum by dialysis against carbowax. The working titre of the conjugate was taken as the highest dilution in buffered saline (pH 7·2) which after incubation with four parts of non-syphilitic serum gave definite (++) fluorescence of treponemes.

Test Procedure

A 2 mm. loopful of a suspension of *T. pallidum* in TPI medium containing about thirty to forty treponemes per high dry field was spread within a 1 cm. diameter circle inscribed on a slide with a diamond, allowed to dry in the air and fixed in fresh acetone for 10 minutes. 0·05 ml. of a mixture of 0·1 ml. inactivated serum plus 0·025 ml. conjugate diluted to its working titre in phosphate buffered saline pH 7·2 (PBS) was added to the film of treponemes and the slides were incubated in a moist chamber at 36°C. for 30 minutes. The slides were washed for two 5-minute periods in two changes of PBS, rinsed in distilled water to remove excess buffer, and mounted in buffered glycerin. Known positive and negative controls were included in each batch of tests. The slides were read at a magnification of ×500 under dark-ground illumination, using a 4 mm. BG 12 exciter filter and appropriate barrier filters.

Results

The results were expressed as:

Complete blocking Treponemes invisible or barely visible, a definite positive result.

Partial blocking Treponemes faintly fluorescent but less than with negative control serum; an indeterminate result.

No blocking Treponemes showing definite ++ fluorescence equal to that with the negative control serum; a negative result.

Tests were carried out on 394 sera on which TPI and FTA-200 tests had also been performed. The results are analysed in Table I.

The TPI test was reactive in 195 instances; of these sera, 133 (68·2 per cent.) gave positive FTA-200 tests; 94 gave complete blocking and 77 partial blocking, a combined reactivity of 87·7 per cent. compared with the TPI results. The TPI test was negative in 199 instances; 26 sera in this group gave positive FTA-200 tests, almost all of these being weak or borderline results. The inhibition test showed complete blocking by one serum and partial blocking by seven. The information available about these patients is summarized in Table II.

TABLE I

COMPARISON OF THE RESULTS OF THE INHIBITION TEST WITH THE FTA-200 TEST AND THE TPI TEST ON 394 PROBLEM SERA

TPI	FTA-200					
	Positive			Negative		
	Inhibition Test			Inhibition Test		
	Complete Blocking	Partial Blocking	No Blocking	Complete Blocking	Partial Blocking	No Blocking
Positive	76	41	6	15	26	13
Doubtful	3	4	3		6	2
Negative	1	3	22		4	169
Total	80	48	31	15	36	184

TABLE II

CLINICAL AND SEROLOGICAL INFORMATION AVAILABLE ON EIGHT PATIENTS WHOSE SERA GAVE REACTIVE INHIBITION TESTS BUT NEGATIVE TPI TESTS

Blocking	Age (yrs)	Sex	Clinical Information	FTA-200	RPCFT	VDRL
Complete	—	F	WI Old treated yaws	+	—	—
Partial	—	F	Gonorrhoea Latent Syphilis diagnosed	+	+	—
	67	—	Ulcer of arm	+	+	—
	—	F	Pregnant	—	—	+
	25	—	WA Gonorrhoea, no history of yaws	—	+	—
	—	F	No details available*	—	—	—
	65	F	Neurological signs	—	—	—
	58	F	Bad vision since birth	—	—	+

WI = West Indian. WA = West African.

*Tests on a previous specimen of serum from this patient had given a doubtful TPI test and a positive FTA-200 test.

It should be stressed that, in selecting sera for the blocking test, as many as possible were included which had shown discrepancies between the TPI and FTA-200 tests and the group does not represent the usual standard of the FTA-200 test. The results obtained suggest that the sensitivity and specificity of the blocking procedure compare favourably with those of the FTA-200 test, taking the TPI results as a standard for comparison.

An assessment of the performance of the inhibition test with presumed normal sera was made by examining 144 sera from blood donors on which VDRL screening tests had been found negative. No blocking was found with any of these.

The pool of serum used to prepare the conjugate contained both group and specific antibodies against *T. pallidum*. A portion of the conjugate was therefore absorbed with Reiter treponemes to remove the group antibody and so give a reagent with which the ability of sera to block reactivity of the specific antibody alone could be studied. The results of tests on 409 sera with this absorbed conjugate are shown in Table III.

The modified inhibition test showed an overall agreement with the TPI test in 91·2 per cent. of the sera tested compared with a similar figure of 82·4 per cent. for the FTA-200 test. Of the 211 sera which were reactive in the TPI test, 194 (92·2 per cent.) showed complete or partial blocking and 180 (85·3 per cent.) were reactive in the FTA-200 test. Complete blocking was more frequent with sera which gave positive results in the TPI test (*i.e.* 51–100 per cent. specific immobilization) and partial blocking only was seen in more than half the sera which gave doubtful TPI results (21–50 per cent. specific immobilization).

In the group of 198 sera which gave negative TPI tests, 41 were reactive in the FTA-200 test; most of these were doubtful borderline reactions and such sera were deliberately selected so as to assess the performance of the modified inhibition test on this difficult material. 28 of these sera showed no blocking, three complete, and ten partial. There were also two sera giving complete and four producing partial blocking among those which were negative in both the TPI and FTA-200 tests. The information available about the patients concerned is summarized in Table IV.

In four cases treponemal infection had either been diagnosed or was possible on clinical grounds and

TABLE III

RESULTS OF "SPECIFIC" INHIBITION, FTA-200, AND TPI TESTS ON 409 PROBLEM SERA

TPI	FTA-200					
	Positive			Negative		
	Inhibition Test			Inhibition Test		
	Complete Blocking	Partial Blocking	No Blocking	Complete Blocking	Partial Blocking	No Blocking
Positive	123	30	4	5	14	6
Doubtful	5	14	4	1	2	3
Negative	3	10	28	2	4	151
Total	131	54	36	8	20	160

TABLE IV

CLINICAL AND SEROLOGICAL INFORMATION AVAILABLE ON NINETEEN PATIENTS WHOSE SERA GAVE REACTIVE "SPECIFIC" INHIBITION TESTS BUT NEGATIVE TPI TESTS

Blocking	Sera	Clinical Information	FTA-200	FTA-ABS	RPCFT
Complete	1	Contact of syphilis	−	+	−
	1	WI	−	+	−
	2	−	+	ND	+
	1	−	+	ND	−
Partial	1	Latent syphilis, treated	+	+	+
	1	Latent syphilis	+	ND	+
	1	WI ? Old yaws	+	+	+
	2	1 WI	+	+	+
	3		+	+	−
	1		+	ND	±
	1		+	ND	−
	2	Pregnancy (1 WI)	−	ND	+
	1		−	+	−
	1	Donor	−	ND	−

ND = Not done. WI = West Indian.

in a further twelve sera the FTA-ABS or Reiter protein complement-fixation test, or both, were positive, in addition to the demonstration of blocking activity of the serum. It is not possible to reach a conclusion about the specificity of these last results on the rather meagre evidence available, but even if they are classed as non-specific, the results shown in Table III suggest that the modified inhibition technique is slightly more sensitive than the FTA-200 test and that it gives fewer positive results that are not confirmed by the demonstration of immobilizing antibody.

Discussion

Indirect fluorescence tests, such as the FTA-200, afford a sensitive method for the detection of anti-treponemal antibody globulins in a serum, but they can make no distinction between the various types of antibody. For this, removal of group reactive antibody by absorption has to be done and up to now workers have used either Reiter treponemes or extracts of these organisms for this purpose. Recent work by Király, Jobbágy, and Kováts (1967) suggests that the position may be more complicated than hitherto supposed in that absorption by Reiter treponemes may not remove all reactivity with other cultivable spirochaetes. Further studies of the distribution of antibodies in normal sera against a wide range of commensal treponemes are needed to clarify the position. The inhibition procedure offers an alternative approach to the demonstration of specific anti-treponemal antibody and has the advantage that it obviates the need for absorbing individual sera.

Ruczkowska (1965), using unabsorbed conjugates, showed that the titre of conjugates roughly paralleled the immobilization titres of the sera from which they were prepared. The inhibition test was found to equal the sensitivity of the TPI test on 411 sera from patients with untreated syphilis and to be rather more sensitive than the TPI test on 834 sera from patients with treated syphilis; in this latter group the FTA test (at a serum dilution of 1 in 100) had an even higher sensitivity: TPI 49·6 per cent., FTA-100 58·2 per cent., inhibition test 54·2 per cent. No positive reactions were found with any of the tests on sera from a control group of 255 patients who were either healthy or were suffering from diseases other than syphilis.

In the present study the conjugate was prepared from a pool of sera from patients with early syphilis because previous work (Wilkinson and Rayner, 1966) had shown that, at this stage of the disease, specific antibody detected by the FTA-200 test formed a larger proportion of the total than at other stages of the disease. The test was found to be technically easy and quick to perform but success depended on a nice adjustment of the titre at which the conjugate was used if maximum sensitivity was to be achieved. Frank positive (complete blocking) and negative reactions (no blocking) were easy to read, but sera giving partial inhibition were more difficult to assess. About a fifth of the sera fell into this intermediate group, which also included most of the observed discrepancies between the inhibition and TPI tests shown in Tables II and IV. This difficulty of assessing partial degrees of inhibition markedly limits the usefulness of the test.

The TPI test has been used as the standard for comparison. This can be accepted as valid when the TPI test is positive, but a negative TPI result does not rule out treponemal disease with the same degree of certainty because of the late appearance of immobilizing antibody in early syphilis and its absence in a minority of cases of late syphilis of long standing, particularly when treatment, either designed or incidental to some other condition, has been given. Thus, in the Tuskegee study of untreated syphilis (Rockwell, Yobs, and Moore, 1964), the TPI test was found reactive in 91 per cent. and the FTA-ABS test in 97 per cent. of the 91 survivors who were examined when their infections were of 30 years or more duration. Allowing for these limitations of the TPI test, the inhibition procedure was found to be more sensitive than the FTA-200 test in the selected group of sera examined in this study and to give fewer unexplained positive results than the latter test. The number of sera tested by the modified technique was small but testing the ability of sera to block staining of treponemes by conjugated specific antibody seems a logical extension of the original method and to merit further study.

Summary

(1) 394 selected problem sera have been tested by the TPI test, FTA-200 test, and an inhibition test based on the ability of sera containing anti-treponemal antibodies to block staining of *T. pallidum* by fluorescein-conjugated syphilitic serum globulins. The inhibition test was found to be more sensitive than the FTA-200 test on this material and to give fewer unexplained positive results. Tests on 144 presumed normal sera were all negative.

(2) An extension of the test is described in which group antibody was removed from the conjugated syphilitic globulin reagent; this allows the ability of sera to block the union of

specific antibody to be investigated. The results of 409 tests with this modification are presented.

My thanks are due to Dr K. Ll. Rogers of the Blood Transfusion Centre, Edgware, for supplying me with sera from blood donors and to Mr H. L. Ferguson for his technical assistance.

REFERENCES

Betz, A. (1966). *Bull. Wld Hlth Org*, **34**, 154.
Deacon, W. E., and Hunter, E. F. (1962). *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 352.
——, Lucas, J. B., and Price, E. V. (1966). *J. Amer. med. Ass.*, **198**, 624.
Fife, E. H. (1964). "Proc. World Forum on Syphilis and Other Treponematoses, Washington, 1962." U.S. Public Health Service Publication No. 997, p. 254. Washington, D.C.
Hunter, E. F. (1964). *Ibid.*, p. 269.
——, 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.
Nairn, R. C. (ed.) (1962). "Fluorescent Protein Tracing." Livingstone, Edinburgh.
Rockwell, D. H., Yobs, A. R., and Moore, M. B. (1964). *Arch. intern. Med.*, **114**, 792.
Ruczkowska, J. (1965). *Arch. Immunol. Ther. exp.*, **13**, 602.
Wilkinson, A. E., and Rayner, C. F. A. (1966). *Brit. J. vener. Dis.*, **42**, 8.

Le test fluorescent inhibiteur de l'anticorps du tréponème

RÉSUMÉ

(1) 394 sérums sélectionnés ont été testés par le test de l'immobilisation du tréponème, le test 200 fluorescent de l'anticorps du tréponème, et un test inhibiteur basé sur la capacité du sérum contenant des anticorps contre le tréponème d'empêcher la coloration des *T. pallidum* par les globulines du sérum syphilitique conjugées à la fluorescéine. Le test inhibiteur a été prouvé comme étant plus sensible que le test 200 fluorescent de l'anticorps du tréponème sur ce matériel et aussi comme ayant donné moins de résultats positifs inexplicables. Les tests faits avec 144 sérums présumés normaux avaient tous été négatifs.

(2) Une extension du test est décrite où l'anticorps de groupe avait été retiré du réactif contenant la globuline conjugée syphilitique; cela a permis l'étude de la possibilité du sérum d'empêcher l'union de l'anticorps spécifique. Les résultats de 409 tests basés sur cette modification sont presentés.