THE TREPONEMAL COMPLEMENT-FIXATION TESTS IN PROBABLE LATENT SYPHILIS OR BIOLOGIC FALSE POSITIVE REACTIONS*

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The successful application of the Treponema Pallidum Immobilization Test (TPI) (1) to the serodiagnosis of syphilis and to the differentiation between true and biological false positive reactions (BFP) has stimulated an accelerated search for less costly and less complex treponemal tests. Currently, there are at least seven modified treponemal procedures under experimental study and evaluation, of which, the several complement-fixation methods appear to be the most promising as practical serodiagnostic tests for routine laboratory use. Several obvious improvements over the TPI test, in this respect, are as follows: the treponemal complement-fixation test can be learned easily and quickly by the average laboratory technician; five to ten times as many complement-fixation tests can be performed in one day; the technical problems of strict adherence to aseptic technique, chemical cleanliness, treponemal survival and maintenance of rabbit colonies are eliminated; and the cost of a complement-fixation test should be markedly less than a TPI test.

The complement-fixation procedures which have received the most attention in this country are the Treponema Pallidum Complement-Fixation Test (TPCF) described by Portnoy and Magnuson in 1955–56 (2, 3) and the Reiter Protein Complement-Fixation Test (RPCF) which is an outgrowth of the fundamental studies of the Italian serologists and dermatologists during the period since 1941. Some of these studies have been summarized in the English language by D'Alessandro *et al* (4, 5) and Pucinelli (6, 7).

In this laboratory, comparisons between the TPI and TPCF tests have been made with specimens from 394 patients with tentative clinical diagnoses of syphilis or BFP reactions. In addition, a correlation between TPCF results and tentative clinical diagnoses and also the reproducibility of TPCF results have been made. A summary of these experiments constitutes the major part of this publication.

In addition, this report includes preliminary observations on the standardization of antigens prepared from the nonpathogenic cultivatable Reiter treponeme.

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MATERIALS AND METHODS

Specimens: the serums used in this study were prepared in this laboratory from blood specimens submitted for TPI testing. Each serum used in the complement-fixation tests had been stored for periods of one week to four months at −10°C. and each was reheated for 10 minutes at 56°C.; and when necessary, the serum was clarified by centrifugation before testing.

Antigens: TPCF antigens were obtained from the Difco Laboratories or were prepared in our own laboratory. These antigens were prepared and standardized according to methods described by Portnoy and Magnuson (2). The only satisfactory Reiter (RPCF) antigen was supplied by the Difco Laboratories.

Complement-Fixation Tests: the serological procedure of the TPCF and RPCF tests followed exactly the modified one-fifth volume Kolmer Complement-Fixation method using 1½ exact units of complement as described by Portnoy and Magnuson (3). All tests were performed by the same individual and read by him and by a second person.

RESULTS

A. *Comparison of TPI and TPCF tests on the same specimens.*

Sera were prepared from 394 blood specimens collected from sero-positive patients whose status in respect to syphilis was not definite. The results of the TPI and TPCF tests on these specimens are summarized in Table I.

The data in Table I show that the TPI test was positive with 205 specimens (52%) and was negative with 189 specimens (48%). The results of the TPCF test were just reversed, in which 190

TABLE I

Comparative TPI and TPCF Results with Specimens from 394 Clinically Questionable Cases of Syphilis

TPI+	TPI−	TPCF+	TPCF−
205/394 (52)*	189/394 (48)	190/394 (48)	204/394 (52)
TPI+ TPCF+	TPI− TPCF−	TPI+ TPCF−	TPI− TPCF+
182/394 (46)	181/394 (46)	23/394 (6)	8/394 (2)

* *Numerator* indicates the number of specimens giving the reactions indicated at the top of each column. *Denominator* indicates the total number of sera tested. *Numbers in parentheses* indicate per cent.

specimens (48%) gave positive reactions and 204 specimens (52%) gave negative reactions. It is of interest to note that the same reaction was obtained with both tests on 363 of the 394 specimens, an agreement of 92 per cent. One hundred and eighty two sera were positive with both tests and 181 were negative with both tests. The results of the two tests differed with 31 specimens, a disagreement of eight per cent.

Since the specificity and sensitivity of the TPI test have been shown to be quite high with serums from clinically proven latent syphilitics or non-syphilitic individuals, then it might be concluded that the differences in this experiment indicate that the TPCF test is less sensitive than the TPI test in patients with latent syphilis, some of whom had been treated earlier for syphilis. This is supported by the data which show that of the 31 specimens with discordant results, three times as many were TPI positive, TPCF negative as were TPI negative, TPCF positive, 23 and 8 respectively.

B. *Correlation between clinical diagnosis and serologic results.*

To further compare the two technics, the results of the TPI and TPCF tests were correlated with tentative clinical diagnosis of syphilis in 127 patients and of biologic false positive reaction (BFP) in 108 patients. These data are summarized in Table II.

It can be seen in Table II that the TPI test was positive in 77 of the 127 suspected cases of

TABLE II

The Correlation between Clinical Diagnosis and TPI and TPCF Reactions

Clinical Diagnosis—Syphilis		Clinical Diagnosis—BFP	
TPI+	TPCF+	TPI−	TPCF−
77/127 (61)	69/127 (54)	68/108 (63)	75/108 (70)

syphilis (61%) and it was negative in 68 of the 108 suspected biologic false positive reactors (63%). Thus, the results of the TPI test agreed with clinical diagnosis in 145 of the total 235 specimens, an agreement of 62 per cent.

A similar comparison between clinical diagnosis and TPCF results shows the following: 69 positive reactions in 127 suspected cases of syphilis (54% agreement); 75 negative reactions in 108 presumed BFP reactors (70% correlation); an agreement between TPCF results and diagnosis in 144 of the 235 cases, 61 per cent.

There was little difference in the over-all correlations between serologic activity and clinical diagnosis when the two tests were compared. However, the agreement between positive reactions and a diagnosis of syphilis was greater with the TPI than with the TPCF test, and the agreement between negative reactions and a diagnosis of BFP reaction was greater with the TPCF than with the TPI test. These findings would suggest again that the TPCF test may be somewhat less sensitive than the TPI test.

C. *The reproducibility of TPCF tests.*

To determine the reproducibility of the test under standard conditions, studies were undertaken in two ways. First *duplicate* tests were performed *simultaneously* with aliquots of 88 TPCF positive and negative serum specimens *employing two different standardized antigens*; secondly, *duplicate tests* employing 61 TPCF positive and negative serum specimens and *the same antigen* were performed *on different days*, within a 14 day period. The results of these studies are summarized in Table III.

The data in Table III, in part I, show that identical results were obtained with 82 of 88 serums (93%) that were tested simultaneously with two different standard TPCF antigens; the results of duplicate tests with six serums (7%) disagreed. Perhaps the degrees of difference for

these six sera, not shown in the table, are more significant than the per cent of difference. Three disagreements were between doubtful and negative reactions, two were between four plus and one plus reactions, and one was between four plus and negative reactions. The latter three differences were of sufficient magnitude that the serums were retested one or more times and the same differences in reactivity were obtained.

The second experiment on reproducibility is summarized in Table III, part II. Duplicate TPCF tests were performed at different times with aliquots of 61 serums and one standard antigen. Only three disagreements (5%) were noted, all of which were differences between doubtful and negative reactions.

These findings suggest that under certain circumstances the results of TPCF tests may not be reproducible, not only in weakly reactive sera, but also in strongly reactive specimens. Further, that disagreements may be slightly more frequent and of greater magnitude when duplicate tests are performed with different antigens than when the same antigen is employed at different times.

D. *Preliminary studies with Reiter (RPCF) antigen.*

The serologic activities of one Reiter antigen and two standardized TPCF antigens were determined by a checkerboard titration of dilutions of the antigens versus dilutions of a standard TPCF positive serum.

The most interesting observation of this comparative titration may be summarized as follows: employing a serum dilution of 1 to 40, the Reiter antigen showed a titer of 1280+; TPCF antigen No. 1, a titer of 40; and TPCF antigen No. 2, a titer of 20. The Reiter antigen showed a zone reaction in the area of antigen excess, i.e., no fixation of complement, in antigen dilutions 1 to 10 and 1 to 20. The results of the total titration suggested that this Reiter antigen may have been at least 25 times as active as the TPCF antigens.

This hypothesis was tested by examining five negative sera, one strongly positive and six weakly positive sera (all diluted 1–5) with the two TPCF antigens at their standard titers of 1 to 12.5 and 1 to 8, and with the Reiter antigen diluted 1 to 320 and 1 to 640. The Reiter dilutions were from 25 to 80 times greater than the TPCF dilutions. The results obtained are summarized in Table IV.

The following interesting observations may be noted in Table IV.

1. No non-specific reactions occurred with either antigen and the five negative sera.

2. No difference of antigen sensitivity was noted with the strongly positive serum.

3. Variations of antigen sensitivity were noted when weakly positive sera were tested. For example, reactions ranging from strongly positive to negative were obtained with aliquots of one serum that was tested with three different antigens. There was as much variation in antigen sensitivity between the two standard TPCF antigens as there was between TPCF Antigen No. 1 and the Reiter antigen at a dilution of 1 to 320. These data suggest that in spite of apparent similarities between different standard TPCF antigens, there may exist differences between them that appear only when they are tested simultaneously with aliquots of the same weakly positive sera.

The results obtained in this study corroborate

TABLE III

Reproducibility of Duplicate TPCF Tests

I. Duplicate tests performed simultaneously with aliquots of 88 specimens employing two different standard antigens.		
	Agreement	*Disagreement*
	82/88 (93)	6/88 (7)
II. Duplicate tests performed on different days, with aliquots of the same serum specimens, with the same standard antigen.		
	Agreement	*Disagreement*
	58/61 (95)	3/61 (5)

TABLE IV

Comparative Specificity and Sensitivity of Reiter and TPCF Antigens

TPI Tested Sera	Reiter Antigen Diluted		TPCF No. 1 Diluted	TPCF No. 2 Diluted
	1-320	1-640	1-12.5	1-8
5 Negative	−	−	−	−
1 Positive	++++	++++	++++	++++
6 Weakly positive	++++	±	++++	±
	−	−	−	−
	−	−	++++	+++
	++++	+++	++++	+
	±	−	++++	−
	++	+	++++	++++

other unpublished data accumulated in our early efforts to produce and standardize TPCF antigens, namely, that the current method of standardizing antigens for treponemal complement-fixation tests on the basis of a serologic titration with one pooled positive serum and one pooled negative serum is unsatisfactory and unreliable. A supplementary step should include the simultaneous testing of several weakly positive and negative individual human serum specimens.

SUMMARY

1. An evaluation has been made of the Treponema Pallidum Complement-Fixation (TPCF) test using the Treponema Pallidum Immobilization (TPI) test as a control.

2. In comparative studies with the two procedures, employing aliquots of serum specimens from 394 cases of possible latent syphilis or biologic false positive reactors, 92 per cent agreement was obtained. The nature of the disagreements indicates that the TPCF test is less sensitive than the TPI test for specimens obtained from the clinical groups described.

3. Under two different experimental conditions, the TPCF test results were reproducible in 93 and 95 per cent of instances of duplicate testing. Reproducibility of results was less satisfactory if aliquots of sera were tested simultaneously with two different antigens than if such aliquots were tested on different days with the same antigen.

4. Comparative standardization of antigens prepared from the cultivatable Reiter treponeme and from the pathogenic Nichols strain of *Treponema pallidum* indicated that the Reiter antigen is probably much more active serologically than the latter antigen. Supplementary steps in the standardization of the treponemal complement-fixation antigens are suggested.

REFERENCES

1. Nelson, R. A., Jr. and Mayer, M. M.: Immobilization of treponema pallidum in vitro by antibody produced in syphilitic infection. J. Exper. Med., **89:** 369–393, 1949.
2. Portnoy, J. and Magnuson, H. J.: Immunologic studies with fractions of virulent treponema pallidum. J. Immunol., **75:** 348–355, 1955.
3. Portnoy, J. and Magnuson, H. J.: Treponema pallidum complement-fixation (TPCF) test for syphilis. Am. J. Clin. Path., **26:** 313–322, 1956.
4. D'Alessandro, G., Oddo, F. G. and Dardanoni, L.: The antigens of the cultured treponema pallidum (Reiter's Strain) and the antispirochetal antibodies in human syphilis. J. Ven. Dis. Inform., **31:** 314–315, 1950.
5. D'Alessandro, G. and Dardanoni, L.: Isolation and purification of the protein antigen of the Reiter treponeme. Am. J. Syph., Gonor. & Ven. Dis., **37:** 137–150, 1953.
6. Pucinelli, V. A.: Recent advances in the serodiagnosis of syphilis. Am. J. Syph., Gonor. & Ven. Dis., **35:** 340–349, 1951.
7. Pucinelli, V. A.: Plurality of antibodies in syphilitic serum and clinical practice. Brit. J. Ven. Dis., **28:** 184–188, 1952.