Evaluation of tpcf-50 Test And Other TPCF Tests For Syphilis Diagnosis

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In recent years, the *Treponema pallidum* immobilization (TPI) test has become one of the most valuable laboratory aids in resolving problem cases in the diagnosis of syphilis. Because of its expense and complexity, however, its use has always been limited to a few large, central laboratories. The phenomenon of immobilization of *T. pallidum* in the presence of treponemal antibody and complement was first used in a serologic test for syphilis described by Nelson in 1949 (*1*). Since then, there have been continuing efforts by many workers to devise other procedures employing the *T. pallidum* as an antigen in serologic tests which might be more simply and rapidly performed, and which might serve as substitutes for the TPI test. Demonstration of an antibody in syphilitic serum which would agglutinate suspensions of killed *T. pallidum* (*2*), and the immune adherence phenomenon (*3*) resulted in the development of serologic tests (*4–7*) which used the whole, killed treponeme as an antigen. One of the most recent promising developments has been the application of fluorescent antibody techniques to the detection of treponemal antibodies (*8*).

The use of an extract of the *T. pallidum* as an antigen in a conventional complement fixation test was described by Portnoy (*9*) in 1955. This sodium desoxycholate extract was used in a modified one-fifth volume Kolmer test and was designated as the *Treponema pallidum* complement fixation (TPCF) test (*10*). The procedure (TPCF I) and an experimental modification (TPCF II) were entered and performed by Portnoy in the Serology Evaluation and Research Assembly (SERA) Study (*11*). A second modification, referred to as the "tpcf-50" test, has recently been described by the same author (*12*), and is referred to as the preferred method, because of increased specificity and economy of time and reagents.

This report presents the comparative results obtained with the three *Treponema pallidum* complement fixation tests and the *Treponema pallidum* immobilization (TPI) test on serums from donors in selected patient categories.

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Materials and Methods

A serum bank has been established at the Venereal Disease Research Laboratory, Communicable Disease Center, Chamblee, Ga., to facilitate evaluation of new serologic tests for syphilis or modifications of published methods. This bank is composed of serums from clinically categorized donors, and includes residuals from approximately 1,200 specimens which were included in the SERA study, in addition to serums from other sources. Convenient aliquots of unheated serum from these selected donors are stored in the frozen state and are drawn from the bank as needed.

The serums are classified in the following donor categories:

PRESUMED NONSYPHILITIC

- Apparently healthy donors presumably with no history of previous or present infection with syphilis.

SYPHILITIC

- Donors with primary syphilis proved by darkfield examination, who had not received treatment.
- Patients having had primary syphilis proved by darkfield examination and who had adequate treatment with 2,400,000 units or more of penicillin not less than 2 nor more than 4 years prior to time blood was taken.
- Donors with secondary syphilis proved by darkfield examination, who had not been treated.
- Patients with secondary syphilis proved by darkfield examination who had adequate treat-

ment with 2,400,000 units or more of penicillin not less than 2 nor more than 4 years prior to time blood was taken.

- Donors with latent syphilis, adequately treated with 4,800,000 units of penicillin.
- Donors with clinical manifestations of late syphilis, such as paresis, tabes, aortic insufficiency, and aneurysm, adequately treated with 4,800,000 units or more of penicillin.
- Donors with late asymptomatic neurosyphilis or unspecified type of neurosyphilis, adequately treated with 4,800,000 units or more of penicillin.
- Donors with manifestations of late syphilis such as aortitis or unspecified type of cardiovascular syphilis, adequately treated with 4,800,000 units or more of penicillin.

With Conditions Other Than Syphilis

- Hospital patients with a variety of diseases or conditions, not receiving antibiotics and having no history or clinical evidence of syphilis.
- Patients 12 years of age or younger with yaws.
- Patients with pinta, below age at which associated syphilis might be expected.
- Leprosy patients not thought to have associated syphilis.

Biologic False Positives

- Patients with reactive nontreponemal tests, at least one nonreactive TPI test, and no clinical evidence of syphilis.
- Patients with reactive nontreponemal tests with no clinical evidence of syphilis and who had no previous TPI test.

Complement Fixation Tests

The TPCF I and TPCF II tests were performed according to the techniques described in the SERA study (*11*), and testing was accomplished at the Venereal Disease Experimental Laboratory, Chapel Hill, N.C.

The tpcf-50 test was performed at the Venereal Disease Research Laboratory in Chamblee, Ga., in accordance with the method described in the Manual of Serologic Tests for Syphilis, 1959 (*13*). Antigen for this test was furnished by the test author.

The TPI test was performed at the Venereal Disease Research Laboratory as described in the manual and was also referred to as "TPI–200" in the SERA study (*11*) conducted by the Public Health Service.

The TPCF I, TPCF II, and the TPI results on 1,208 SERA study specimens were taken from the SERA study report (*11*). The tpcf-50 test was performed at a later date at this laboratory on residuals of these same serums which had not been previously heated or tested and had been stored in the frozen state in tightly sealed containers since the original date of collection and separation of serum. The numbers of these specimens were coded so that the testing activity had no prior knowledge of the results obtained with the tests previously performed in the SERA study. An additional 263 specimens in the presumed nonsyphilitic category, which had not been included in the SERA study, were also tested in the tpcf-50 test. The TPI test was performed on all specimens in this group which were not nonreactive with the tpcf-50 test.

Results

The results obtained with the three *Treponema pallidum* complement fixation tests and the TPI test on 326 presumed nonsyphilitic donors are shown in table 1. The reactivity rates of the TPCF I (13.5 percent) and the tpcf-50 (13.8 percent) tests were almost identical, but were almost five times as great as were obtained with the TPI test (2.76 percent). The tpcf-50 test was also performed on an additional 263 specimens in this donor category, which were not included in the SERA study and reactive results were obtained in 15 in-

Table 1. Results of the TPCF tests and TPI test on presumed nonsyphilitics

Test	Nonreactive		Reactive [1]	
	Number	Percent	Number	Percent
TPCF I	282	86.50	44	13.50
TPCF II	256	78.53	70	21.47
tpcf-50	281	86.20	45	13.80
TPI	317	97.24	9	2.76

[1] Including weakly reactive.

Table 2. TPCF and TPI test results obtained on 477 serums from patients in eight categories of syphilis

Category	Number of specimens	TPCF I		TPCF II		tpcf-50		TPI	
		Reactive [1]		Reactive [1]		Reactive [1]		Reactive [1]	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Primary, untreated	119	80	67. 23	84	70. 59	87	73. 11	45	37. 82
Primary, treated	29	11	37. 03	15	51. 72	16	55. 17	6	20. 69
Secondary, untreated	84	82	97. 62	83	98. 81	83	98. 81	82	97. 62
Secondary, treated	18	11	61. 11	16	88. 89	16	88. 89	11	61. 11
Latent, treated	24	20	83. 33	22	91. 67	19	79. 17	22	91. 67
Late, treated:									
With clinical manifestations	63	54	85. 71	56	88. 89	55	87. 30	62	98. 41
With asymptomatic or unspecified neurosyphilis	112	105	93. 75	105	93. 75	102	91. 07	109	97. 32
With aortitis or unspecified cardiovascular	28	21	75. 00	19	67. 86	23	82. 14	26	92. 86

[1] Including weakly reactive.

stances. The TPI test, performed on these tpcf-50 reactive serums, was reactive in two instances, weakly reactive in 1, and nonreactive in the other 12.

In primary syphilis, the tpcf-50 test was the most reactive of the treponemal complement fixation tests, and the TPI test was the least reactive of the four procedures (table 2). In untreated secondary syphilis, all four tests showed a high degree of reactivity, but in treated secondary syphilis, the TPCF II and the tpcf-50 tests were considerably more reactive than either the TPCF I and TPI tests, which gave identical findings. In latent and late syphilis, the tpcf-50 and TPCF I tests were in close agreement but were consistently less reactive than the TPI test.

Results obtained in four categories of diseases other than syphilis showed the tpcf-50 test to be in closer agreement with the TPI than was the TPCF I test of hospital patients with a variety of diseases having no history or clinical evidence of syphilis (table 3). In yaws and pinta, all four tests were 90 to 100 percent reactive. In a group of 29 patients with leprosy, one reactive result was obtained with both the tpcf-50 and TPCF I tests and two with the TPCF II modification. No reactive results were observed in this group with the TPI test.

Table 3. TPCF and TPI test results obtained from donors with diseases or conditions other than syphilis

Category	Number of specimens	TPCF I		TPCF II		tpcf-50		TPI	
		Reactive [1]		Reactive [1]		Reactive [1]		Reactive [1]	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Variety of conditions in hospital patients	73	8	10. 96	11	15. 07	6	8. 22	5	6. 85
Yaws	36	35	97. 22	35	97. 22	36	100. 00	36	100. 00
Pinta	50	46	92. 00	46	92. 00	46	92. 00	47	94. 00
Leprosy	29	1	3. 45	2	6. 90	1	3. 45	0	0. 00

[1] Including weakly reactive.

Table 4. TPCF and TPI test results obtained from donors classified as biological false positive reactors

Category	Number of specimens	TPCF I		TPCF II		tpcf-50		TPI	
		Reactive [1]		Reactive [1]		Reactive [1]		Reactive [1]	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Nontreponemal test, reactive, TPI nonreactive	111	11	9. 91	14	12. 61	10	9. 01	5	4. 50
Nontreponemal test reactive, no clinical evidence	109	444	40. 37	47	43. 12	34	31. 19	38	34. 86

[1] Including weakly reactive.

All of two groups of donors classified as biological false-positive reactors were reactive in one or more nontreponemal tests in the absence of any clinical or anamnestic evidence of syphilitic infection (table 4). In a group limited to patients who were previously nonreactive in at least one TPI test, almost identical reactivity rates were obtained with the TPCF I and tpcf-50 tests (9.91 percent and 9.01 percent, respectively), but these were approximately twice that of the TPI test (4.05 percent). Although it might be expected that the TPI test would be completely nonreactive in this group because of the method of preselection of donors, the five TPI reactions obtained among these patients could be attributed to the fact that the procedure used in screening these patients may have been less reactive than the modification used in this study. Where the biological false-positive diagnoses were made by clinical impression without previous TPI test screening, all four tests were more reactive than among those who were not reactive to the TPI test, possibly due to the presence of syphilis, treated or untreated, in this group of donors. The TPI and tpcf-50 tests, however, were in closer agreement than the other two tests, in this last category.

Summary

The three TPCF tests and the TPI test were used on serums from donors in selected categories and results compared.

1. In the presumed nonsyphilitic group, the tpcf-50 test and the original TPCF were in close agreement. However, the reactivity rate of both tests was five times greater than that of the TPI test.

2. In primary and secondary syphilis, the tpcf-50 test was either comparable to or more reactive than the TPCF I and TPI tests, but in latent and late syphilis, with the exception of neurosyphilis, all three TPCF tests were consistently less reactive than the TPI test.

3. In diseases other than syphilis, excluding the treponemal diseases yaws and pinta, the TPI test gave fewer reactions than the three TPCF procedures.

4. In the group of donors preselected by at least one nonreactive TPI test, approximately 5 percent (5 of 106) of the patients who were nonreactive with the TPI test in this study were reactive in the tpcf-50 test.

5. In the group of donors diagnosed as biological false-positive reactors by clinical impression and without previous screening with a TPI test, the reactivity rate was approximately the same with the tpcf-50 and TPI tests.

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Research Support by Foundations and Health Agencies

During 1957, $95 million was spent for scientific research and development by private philanthropic foundations and voluntary health agencies in the United States, the National Science Foundation reports. Of 4,067 private foundations surveyed, 438 reported research and development programs and a total outlay of $72 million. Twenty-five of 30 voluntary health agencies reported expenditures of $23 million.

Basic research received $59 million of the total spent. Private foundations gave major support to the life sciences, about 45 percent of their total expenditures; social sciences were next in dollar support; physical sciences received the least. Almost one-half of the expenditures reported by the voluntary health agencies were to support biological and medical research.

Most of the expenditures were in the form of grants to outside organizations and individuals. Educational institutions and affiliated medical schools and hospitals were the major recipients.

These and other findings are a summary of preliminary data compiled for the National Science Foundation by the Bureau of Labor Statistics of the U.S. Department of Labor and published under the title "Research and Development Expenditures of Foundations and Health Agencies, 1957" as No. 15 of the Foundation's series "Reviews of Data on Research and Development."