TPI and TPCF Tests On 2,000 Patients Difficult to Diagnose

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SINCE January 1, 1955, the Venereal Disease Research Laboratory of the Public Health Service has offered a *Treponema pallidum* immobilization (TPI) testing service on a nationwide basis to all physicians through the laboratories of State and Territorial departments of health. The Venereal Disease Research Laboratory has received from State laboratories requests for TPI testing of approximately 100 serums per week since commencement of the service (*1, 2*). During a part of this period, the *Treponema pallidum* complement fixation (TPCF) test also was used—experimentally and as a research tool. From July to December 1956, both these procedures were used on all serums submitted for TPI testing, and in January 1957 the TPCF test was made an integral part of the TPI testing service.

All serums submitted for TPI testing have been tested first with the TPCF procedure since January 1957. Only those serums that are not nonreactive in the TPCF test are then tested with the TPI procedure. When both tests are performed, the completed report identifies each test by name and lists the results obtained in each.

This report presents analyses of results obtained with TPI and TPCF tests on 2,000 serums tested during the period from July to December 1956, when all serums were being tested with both procedures, and relates these findings to the patient data and medical opinions submitted with the serums. Serums were received from more than 1,300 physicians in 47 States and Territories, so they probably represent a fair cross section of the diagnostic-problem patients of the average physician. The patients included 1,255 females (62.75 percent) and 745 males (37.25 percent). This percentage distribution by sex is approximately the same as has been noted since the TPI testing service was introduced, and it may indicate that the diagnostic problem in regard to syphilis is occurring approximately twice as often in female as in male patients (*3*).

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Methods

Serums. Specimens submitted for TPI testing service are received from the State and Territorial departments of health laboratories, accompanied by completed clinical data sheets. The clinical data sheet, in addition to identifying the patient, lists blood and spinal fluid test reports, any history of treponematoses and treatment given, history of several conditions or infections known to be associated with biologic false-positive (BFP) reactions, and finally, the opinion of the attending physician as to the probable diagnosis of the patient at the time the blood is submitted. The stipulations for the acceptance of specimens for this service are that they be sterile serums from diagnostic-problem patients with no history or clinical evidence of syphilis or with suggestive evidence of untreated syphilis.

TPI Test. The TPI test was performed according to the Nelson technique (*4, 5*) as modified in later publications by the Venereal Disease Research Laboratory (*6–9*). Only those serums which, after treatment with penicillinase, produced less than 70 percent motility in the control tube were reported as inconclusive.

TPCF Test. The qualitative TPCF test was performed according to the technique described by Portnoy and Magnuson (*10*). Antigen was obtained from the Venereal Disease Experimental Laboratory, Chapel Hill, N. C. An anticomplementary report was recorded on those specimens that were anticomplementary in qualitative testing and insufficient in quantity to allow preparation of dilutions for retesting as prescribed in the technique.

Discussion

Specific test results obtained with the TPI and TPCF tests on 2,000 serums (745 from male patients and 1,255 from female patients) are shown in table 1. Several observations may be made from these data. The TPCF test was slightly more reactive for this donor group than was the TPI test. Although 42.5 percent were reactive to the TPCF, while 47.7 percent were reactive to the TPI test, the reactive plus weakly reactive results totaled 51.0 percent for the TPCF and only 49.8 percent for the TPI test. Conversely, the percentage of nonreactive findings was greater with the TPI test (50 percent) than with the TPCF test (48 percent). The percentage of nonreactive results in both tests was higher for serums from females (TPI 54.4 percent and TPCF 51.2 percent) than for the specimens from males (TPI 42.6 percent and TPCF 42.7 percent). As indicators of probable false-positive reactions in other tests for syphilis, the nonreactive results in these tests suggest that the proportion of probable biologic false-positive reactors is higher in female than in male patients.

Only 20 (1 percent) of the 2,000 serums were reported as anticomplementary in the TPCF test (see "Methods") and only 3 serums (0.2 percent) were reported inconclusive in the TPI test.

Actual agreement between the results obtained with the TPI and TPCF tests is not as great as is indicated by the total percentage of reactive plus weakly reactive results for the two tests. One hundred and one serums (5.0 percent) that were nonreactive in the TPCF test produced reactive or weakly reactive results in the TPI test. One hundred and thirty-one serums (6.5 percent) that produced nonreactive results in the TPI test were either reactive or weakly reactive in the TPCF test. Direct disagreement of results obtained with the two tests was produced, therefore, in 11.5 percent of the 2,000 specimens. Inclusion of anticomplementary and inconclusive findings brings the nonagreement total to 12.7 percent.

Complete serologic agreement of these two tests was obtained in 1,745 (87.3 percent) of the serums in the series. Both tests were reactive or weakly reactive in 887 (44.4 percent), and both were nonreactive in 858 (42.9 percent) of the specimens tested.

Since the TPCF test is now being used as a screening procedure for the TPI testing service, the 101 serums showing nonreactive results in the TPCF test but with some degree of reactiv-

Table 1. Comparative results of TPI and TPCF tests on 2,000 serums

TPCF Test: Type of reaction	TPI test: Total		Reactive		Weakly reactive		Nonreactive		Inconclusive	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Both sexes	2,000	100.0	956	47.7	41	2.1	1,000	50.0	3	0.2
Reactive	851	42.5	781	39.0	8	.4	61	3.0	1	.1
Weakly reactive	169	8.5	93	4.6	5	.3	70	3.5	1	.1
Nonreactive	960	48.0	74	3.7	27	1.3	858	42.9	1	.1
Anticomplementary	20	1.0	8	.4	1	.1	11	.6	-------	-------
Males	745	100.0	417	56.0	10	1.3	317	42.6	1	.1
Reactive	364	48.9	344	46.2	2	.3	18	2.4	-------	-------
Weakly reactive	56	7.5	37	5.0	1	.1	18	2.4	-------	-------
Nonreactive	318	42.7	35	4.7	6	.8	276	37.1	1	.1
Anticomplementary	7	.9	1	.1	1	.1	5	.7	-------	-------
Females	1,255	100.0	539	43.0	31	2.5	683	54.4	2	.1
Reactive	487	38.8	437	34.8	6	.5	43	3.4	1	.1
Weakly reactive	113	9.0	56	4.5	4	.3	52	4.1	1	.1
Nonreactive	642	51.2	39	3.1	21	1.7	582	46.4	-------	-------
Anticomplementary	13	1.0	7	.6	-------	-------	6	.5	-------	-------

Table 2. Medical data supplied with 101 serums nonreactive in TPCF and reactive or weakly reactive in TPI tests

Diagnostic category and sex	Total	History of syphilis	No history of syphilis
All diagnostic categories	101	24	77
Males	41	9	32
Females	60	15	45
Syphilis	18	12	6
Males	9	5	4
Females	9	7	2
Biologic false positive	71	9	62
Males	28	3	25
Females	43	6	37
None stated	12	3	9
Males	4	1	3
Females	8	2	6

ity in the TPI test are of most interest. Table 2 shows that these serums were from 18 patients considered to be syphilitic, 71 patients considered to be biologic false-positive reactors, and 12 patients for whom no clinical opinion had been given. The group includes 24 patients with some history of syphilitic infection and 77 patients without positive history or physical findings of infection. Distribution in this group as to sex, history of previous syphilitic infection, and present diagnosis is not unlike that in the remainder of the 2,000 patients in this study. One significant difference noted of this group, however, was the number of weakly reactive TPI test results. Twenty-five (25 percent) of these 101 serums produced weakly reactive TPI test findings as compared with only 41 (2 percent) of the total 2,000 serums tested.

Seventeen hundred and fifty-four of the serums were accompanied by data sheets indicating the clinical impression of the submitting physician as to whether the patient was a biologic false-positive reactor or had syphilis. Comparisons of test results obtained on specimens in these two categories are presented in tables 3 and 4. No clinical opinion was indicated on the data sheets for the remaining 246 serums.

Of the 1,407 serums from patients listed as probable biologic false-positive reactors (table 3), 47 percent were nonreactive to both the TPI and TPCF tests. Results from either test alone were in slightly closer agreement with the medical opinion that these specimens were from BFP reactors, since 54.3 percent nonreactive results were obtained with the TPI test and 52.0 percent were produced in the TPCF test. Of the 1,407, 40.3 percent were either reactive or weakly reactive in both the TPI and TPCF tests. The group of specimens from females showed closer agreement with medical opinion

Table 3. Comparison of TPI and TPCF test results on 1,407 serums from patients diagnosed biologic false-positive reactors

	Type of reaction	TPI Test							
		Total		Reactive or weakly reactive		Nonreactive		Inconclusive	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
TPCF Test	Both sexes	1,407	100.0	643	45.6	762	54.3	2	0.1
	Reactive or weakly reactive	661	47.0	567	40.3	92	6.6	2	.1
	Nonreactive	732	52.0	71	5.0	661	47.0	----	----
	Anticomplementary	14	1.0	5	.3	9	.7	----	----
	Males	492	100.0	268	54.5	224	45.5	----	----
	Reactive or weakly reactive	263	53.5	239	48.6	24	4.9	----	----
	Nonreactive	224	45.5	28	5.7	196	39.8	----	----
	Anticomplementary	5	1.0	1	.2	4	.8	----	----
	Females	915	100.0	375	41.0	538	58.8	2	.2
	Reactive or weakly reactive	398	43.5	328	35.9	68	7.4	2	.2
	Nonreactive	508	55.5	43	4.7	465	50.8	----	----
	Anticomplementary	9	1.0	4	.4	5	.6	----	----

Table 4. Comparison of TPI and TPCF test results on 347 serums from patients diagnosed as syphilitic

TPCF Test: Type of reaction	TPI Test: Total		Reactive or weakly reactive		Nonreactive		Inconclusive	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Both sexes	**347**	**100. 0**	**233**	**67. 2**	**113**	**32. 5**	**1**	**0. 3**
Reactive or weakly reactive	237	68. 3	214	61. 7	23	6. 6		
Nonreactive	108	31. 1	18	5. 2	89	25. 6	1	. 3
Anticomplementary	2	. 6	1	. 3	1	. 3		
Males	**164**	**100. 0**	**109**	**66. 5**	**54**	**32. 9**	**1**	**. 6**
Reactive or weakly reactive	110	67. 1	100	61. 0	10	6. 1		
Nonreactive	53	32. 3	9	5. 5	43	26. 2	1	. 6
Anticomplementary	1	. 6			1	. 6		
Females	**183**	**100. 0**	**124**	**67. 7**	**59**	**32. 3**		
Reactive or weakly reactive	127	69. 4	114	62. 3	13	7. 1		
Nonreactive	55	30. 1	9	4. 9	46	25. 2		
Anticomplementary	1	. 5	1	. 5				

than specimens from males. Nonreactive results were produced by TPI in 58.8 percent, and by TPCF in 55.5 percent of the serums from female donors, whereas each test produced nonreactive results in 45.5 percent of the serums from males. This may reflect some factor, present in the female, that is responsible for an increased percentage of BFP reactions.

Of the 347 specimens from patients currently diagnosed as syphilitic (table 4), 61.7 percent were reactive or weakly reactive in both tests, while 25.6 percent were nonreactive in both tests. Again slightly closer agreement with medical opinion was shown by the results of each test considered independently, since 67.2 percent reactive results were produced in the TPI test and 68.3 percent were obtained in the TPCF test. Here no significant difference between reactivity in either test of serums from male and female donor groups was noted.

Approximately 5 percent of the serums in this study produced TPI reactions (reactive or weakly reactive) and would have been missed if the TPCF nonreactive serums had not been tested further. However, 6.5 percent of the serums were reactive or weakly reactive in the TPCF test and produced nonreactive findings in the TPI test. It is estimated from these bases that the TPCF test, when used as a screening procedure for the TPI test, may detect (including anticomplementary findings) approximately 90 percent of the serums that will produce reactive or weakly reactive findings in the TPI test, although a larger total number of reactive plus weakly reactive TPCF test results may be obtained. The percentage of agreement between results obtained with the TPI and TPCF tests reported here would not necessarily be similar to findings produced by testing groups of specimens selected in a different manner.

Summary

Results obtained with the TPI and TPCF tests on 2,000 serums from diagnostic-problem patients were in agreement on 1,745 serums (87.3 percent).

The greater proportion of nonreactive results produced by serums from female patients in both or either of the treponemal tests is an indication that biologic false-positive reactions may be more prevalent among female patients.

REFERENCES

(*1*) Harris, A., and Bossak, H. N.: Serology control program of the Venereal Disease Research Laboratory. Pub. Health Rep. 72: 142–144, February 1957.

(*2*) Bossak, H. N., Falcone, V. H., and Harris, A.:

A year's experience with a nationwide TPI testing service. Pub. Health Rep. 72: 317–320, April 1957.

(3) Harris, A.: TPI test as a daily routine laboratory procedure. Am. J. Pub. Health 46: 723–727 (1956).

(4) 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).

(5) Nelson, R. A., Jr., and Diesendruck, J. A.: Studies on treponemal immobilizing antibodies in syphilis. I. Technique of measurement and factors influencing immobilization. J. Immunol. 66: 667–685 (1951).

(6) Portnoy, J., Harris, A., and Olansky, S.: Studies of the *Treponema pallidum* immobilization (TPI) test. I. The effect of increased sodium thioglycollate and complement. Am. J. Syph., Gonor. & Ven. Dis. 37: 101–105 (1953).

(7) Ajello, G., Portnoy, J., Logan, L., and Olansky, S.: Studies of the *Treponema pallidum* immobilization (TPI) test. IV. A simplified method of preparing the modified basal medium. Am. J. Syph., Gonor. & Ven. Dis. 38: 288–294 (1954).

(8) Harris, A., Bossak, H. N., and Olansky, S.: Laboratory aspects of the *Treponema pallidum* immobilization (TPI) test. Pub. Health Lab. Bull. 13: 63–66 (1955).

(9) Bossak, H. N., Harris, A., and Olansky, S.: Studies of the *Treponema pallidum* immobilization (TPI) test. V. The effect of blood-collecting tubes. Pub. Health Lab. Bull. 12: 153–157 (1954).

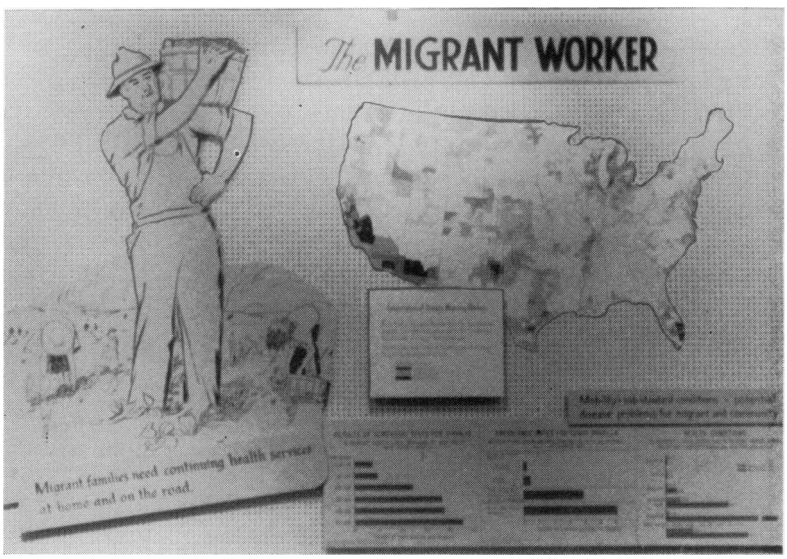
(10) Portnoy, J., and Magnuson, H. J.: *Treponema pallidum* complement-fixation (TPCF) test for syphilis. Am. J. Clin. Path. 26: 313–322 (1956).

PHS exhibit

The Domestic Migrant Worker

To encourage community effort in meeting the health problems of domestic agricultural migrants on a continuing basis, information is supplied through a Public Health Service exhibit.

The exhibit consists of maps, charts, and photographs, illustrating the major routes traveled, important work areas, typical housing, and health conditions. It gives examples of organized effort in making health services available to the migrants who follow the crops each year. The exhibit is designed for display at national, regional, and State conferences, and in other places where interested people gather together.



Specifications: A 3-panel exhibit on legs, 7 feet high, total weight 377 lbs., including the packing crate; back panel, 4′ x 6′; each of the side panels, 4′ x 3′, attached to the center by hinges. Lighting fixtures fit into each of the panels through slots at the top, and the three 150-watt reflector floodlights can be connected with a single outlet. Available through the Division of General Health Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Washington 25, D. C.