A Year's Experience With a Nationwide TPI Testing Service

By Hilfred N. Bossak, B.S.,
Virginia H. Falcone, B.S.,
and Ad Harris

SINCE THE ADVENT of the *Treponema pallidum* immobilization (TPI) test, laboratories throughout the world have been using this test procedure either experimentally as a research tool or as a diagnostic aid. However, numerous modifications of the original Nelson and Mayer method (*1*) have been adopted. A World Health Organization report (*2*) containing information from 25 laboratories performing the TPI test indicates little uniformity among the techniques employed.

Previous reports from the Venereal Disease Research Laboratory (*3, 4*) have shown that the reactivity level of the TPI test may be affected by alteration of the complement concentration in the test, by an increase in the amount of thioglycolate in the survival medium, or by failure to maintain an adequate and constant number of organisms in the treponeme suspension used as the test antigen. The technique employed at the laboratory during the period of this report used a concentration of complement four times greater than that recommended in the original Nelson and Mayer procedure, a fivefold increase in the concentration of thioglycolate, and a constant concentration of 15 organisms per high-dry (HD) field. As a result of the adoption of these and other selected modifications (*5, 6*) the TPI test has been in daily operation in the Venereal Disease Research Laboratory as a routine procedure for more than 18 months, with test runs including as many as 60 specimens per day.

In response to increasing requests for performance of the TPI test from physicians throughout the United States and in the absence of adequate facilities for performing this procedure elsewhere, a TPI testing service was made available by the Venereal Disease Research Laboratory on a nationwide basis. Beginning January 1, 1955, State and Territorial departments of health laboratories and Public Health Service installations were included.

The purpose of this report is to evaluate a 1-year experience with this nationwide service, and to present the findings obtained by testing more than 2,400 serum specimens submitted through the State and Territorial departments of health laboratories.

During the period January 1 to December 31, 1955, a total of 2,875 serums were received from these sources. Of these, 2,487 were tested, 150 were bacterially contaminated and were unsatisfactory for testing, 15 had leaked or the tubes had been broken in transit, and 223 were not tested until after December 31, 1955, and therefore were not included in this report. The number of specimens submitted for testing increased sharply as the availability of the service was more widely publicized. During the first 6 months, 704 specimens were received. However, during the next 3 months more than 1½ times as many, or 1,140 serums, were received for testing. During the last 3 months of the year approximately the same number, namely, 1,031 specimens, were submitted for testing. The totals for the last 6 months of the year represented an actual testing load of 350 to 400 specimens per month.

Source of Specimens

Although serum specimens from other sources were tested during this period, only those from the laboratories of State departments of health are referred to in this report since each of these specimens was accompanied by a clinical data sheet listing pertinent facts of the patient's history. The data included evidence of history of treponematosis, such as previous diagnosis, therapy for syphilis, and evidence of syphilitic infection in the family; record of known serologic tests for syphilis; evidence of other diseases, especially those presumed to elicit nonspecific reactions in serologic tests for syphilis; and the physician's opinion

Mr. Bossak and Mrs. Falcone are bacteriologists and Mr. Harris is director, Venereal Disease Research Laboratory, Public Health Service, Chamblee, Ga.

about the present condition of the patient in terms of probable syphilitic infection. The bases for medical opinions were probably not constant since the 2,875 specimens were submitted by several hundred physicians through more than 50 State and Territorial laboratories.

Criteria for Acceptance

Criteria for acceptance in the TPI testing service required that the specimen be obtained from either diagnostic problem patients with no history or clinical evidence of syphilis or patients with suggestive evidence of syphilitic infection who had not received treatment; that neither the blood nor serum be exposed to rubber stoppers; and that a sterile serum sample be prepared by the State or Territorial health department laboratory and submitted with a completed clinical data sheet in the same mailing container to the Venereal Disease Research Laboratory. Specimens received directly from physicians were returned without testing. Reports of TPI test results were returned only to the submitting laboratory for subsequent forwarding to the originating source. The Venereal Disease Research Laboratory has furnished guide forms explaining these requirements, together with clinical data sheets, to the State public health laboratories for distribution to physicians.

Valid results were obtained with the TPI test on 2,465 serum specimens (table 1). The terms "reactive," "weakly reactive," and "nonreactive" are used instead of "positive," "weakly positive," or "doubtful," and "negative," in accordance with the 1953 recommendations of the National Serology Advisory Council to the Surgeon General of the Public Health Service. Table 2 summarizes the findings for all of the 2,652 specimens which were included in this study.

All patients whose serum produced an inconclusive result in this test were treated with penicillinase and the serums were retested. The low percentage of inconclusive reports (less than 1 percent) was attributed to the use of paraffin-coated cork-stoppered tubes (*7*) instead of the rubber-stoppered tubes used in previous studies (*8*). Only 0.6 percent of the specimens were lost through leakage or breakage in transit. However 150 specimens, or almost 6 percent of the total, were grossly bacterially contaminated upon arrival and so were not tested. They were reported as "unsatisfactory for testing, due to bacterial contamination." Since adequate evidence regarding the possible effect of bacterial contamination of the serum on the TPI test result was not available, it was considered to be inadvisable to test grossly contaminated specimens.

As shown in table 1, approximately two-thirds of the specimens were from female patients. Since a request for a TPI test probably indicates some doubt as to syphilitic infection, these figures indicate that diagnostic problems occurred more frequently in females.

For the serum specimens which produced valid TPI test results, the percentage of positive findings (reactive plus weakly reactive) was lower for female donors (41.7 percent) than for male donors (52.4 percent). If TPI test negativity is taken as an index of false-positive reactions in other tests for syphilis, then these findings indicate a higher percentage of biologic false-positive reactors among female patients than was encountered among the male patients in this selected group. It

Table 1. Results of TPI tests on 2,465 serums submitted to the Venereal Disease Research Laboratory by State and Territorial health department laboratories, January 1–December 31, 1955

Test result	Male		Female		Total	
	Number	Percent	Number	Percent	Number	Percent
Reactive	453	50. 3	633	40. 5	1, 086	44. 1
Weakly reactive	19	2. 1	19	1. 2	38	1. 5
Nonreactive	428	47. 6	913	58. 3	1, 341	54. 4
Total	900	100. 0	1, 565	100. 0	2, 465	100. 0

Table 2. Summary of 2,652 serums submitted to the Venereal Disease Research Laboratory by State and Territorial health departments, January–December 1955

Test results	Male		Female		Total	
	Number	Percent	Number	Percent	Number	Percent
Valid	900	33. 9	1, 565	59. 0	2, 465	92. 9
Inconclusive	8	0. 3	14	0. 5	22	0. 8
Not tested:						
Bacterially contaminated, unsatisfactory for testing	45	1. 7	105	4. 0	150	5. 7
Leaked or broke in transit	5	. 2	10	. 4	15	. 6
Total	958	36. 1	1, 694	63. 9	2, 652	100. 0

Table 3. Diagnosis and history versus TPI test result on 2,449 serums submitted to the Venereal Disease Research Laboratory, January 1–December 31, 1955

Current diagnosis	Number	Percent	History	TPI test result	
				Reactive or weakly reactive	Nonreactive
Syphilis	295	12	Syphilis	103	28
			No syphilis	100	64
Biologic false-positive reaction	1, 772	72	Syphilis	97	40
			No syphilis	621	1, 014
No opinion stated	382	16	Syphilis	66	36
			No syphilis	131	149
Total	2, 449	100		1, 118	1, 331

should be noted, however, that the patients in this study represented diagnostic problem cases, and the relationship observed between TPI test result and sex of the patient is not necessarily representative of random population groups.

Complete data relating diagnoses and patient histories to the TPI test results were available for 2,449 serum specimens (table 3). Of these patients, 1,772, or approximately 72 percent, were considered by the physicians submitting the serum specimens to be biologic false-positive (BFP) reactors in other tests for syphilis. In this group 137 patients had histories of a previous diagnosis of syphilis, but serum from 40 of these patients was nonreactive in the TPI test.

Of the 1,772 serum specimens giving biologic false-positive reactions, 718, or 40.5 percent, produced reactive or weakly reactive results in the TPI test. The positive findings obtained with other serologic tests for syphilis in the absence of clinical evidence of syphilitic infection must be referred to either as latent syphilis or as false-positive test results. As determined from data furnished in the clinical data sheets accompanying the specimen of serum, 38 percent of those meeting these criteria gave reactive results in the TPI test. If it is presumed that a reactive result in the TPI test can only be caused by one of the treponemal infections, past or present, then the TPI test would be 100 percent specific by definition, and in this study the physician's current diagnosis of a biologic false-positive reaction would have been in error 38 percent of the time. On the other hand, as shown in table 3, regardless of the physician's opinion of present status of the patient, 72 percent of the serum specimens from 370 patients with histories of past syphilitic infections gave reactive results in the TPI test. The remaining 28 percent produced nonreactive results and were in disagreement with a previ-

ous diagnosis of syphilis made some time in the patient's past. Of those 295 specimens accompanied by a current diagnosis of syphilis, 92, or 31 percent, failed to react in the TPI test. The percentage of disagreement between a diagnosis of syphilis and the TPI test result was approximately the same, regardless of when the diagnosis was made.

In the biologic false-positive diagnosis category, 60 percent agreement with the TPI test was obtained. Overall correlation between the physician's present clinical opinion and the TPI test was approximately 61 percent.

Summary

1. Modifications of the original Nelson and Mayer method for performance of the *Treponema pallidum* immobilization (TPI) test adopted by the Venereal Disease Research Laboratory have made it possible to perform this test as a daily operating, routine laboratory procedure.

2. During a 1-year period of a nationwide TPI testing service offered to State and Territorial public health laboratories, more than 2,800 serums from diagnostic problem cases in syphilis were submitted for testing.

3. TPI test findings were considered in relation to the sex of patients, evidence of syphilitic infection, past or present, and the clinical opinions of the physicians submitting specimens for testing.

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