Control Serum for the TPI Test

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THE *Treponema pallidum* immobilization (TPI) test described by Nelson (*1,2*) has been modified by many of the laboratories which perform this test (*3*). Controls for the originally described TPI test included a "quantitatively titered syphilitic serum." At a meeting called by the Division of Venereal Disease, Public Health Service, in Washington, D.C., February 1, 1952, to discuss the TPI test, several participants expressed the need for a common, reactive serum, available to all laboratories, by which sensitivity levels of the TPI test could be measured from day to day within a laboratory and also between laboratories. Later that year such a control serum was distributed by the Public Health Service's Venereal Disease Experimental Laboratory to the Venereal Disease Research Laboratory and to other laboratories. This serum was frozen and sent by airmail in packages with solid CO_2.

Experiments conducted in 1952 (*4*) indicated that whole serum, to be used as quantitative reactive controls for the TPI test, could be distributed to distant laboratories in the liquid state without added preservatives or refrigeration. In March 1953 freeze-dried ampoules of the first international TPI control serum were distributed to 25 laboratories by the World Health Organization (*5*), and in February 1954 ampoules of the second international control serum were sent to 35 laboratories. Results of testing with these serums are reported and analyzed in a 1956 WHO report (*6*) in which the following statement is made regarding the use of a quantitative serum in the TPI test:

" In accordance with experiences from studies on reagin tests, the use of a reference (or standard) serum is recommended. In this way the effect of the variations of the titer levels between the laboratories as well as the temporal time variations within laboratories may be reduced."

At the Venereal Disease Research Laboratory a reactive serum has been used as a quantitative control for the TPI test since July 1952. Since then the serum has been available to all TPI testing laboratories in the United States, under the following conditions:

- A single serum is to be common to all TPI testing laboratories, and this serum is to be replaced later by another serum that will be sent to all participating laboratories at the same time. The supply of serum on hand will suffice for at least 2 years.
- All participating laboratories, including the Venereal Disease Research Laboratory, will keep a record of quantitative results obtained with this control serum each time it is used. A report containing these findings will be forwarded to the Venereal Disease Research Laboratory twice each year, in July and in January, where it will be duplicated and forwarded to all other participating laboratories without analysis or comment.

The Venereal Disease Research Laboratory mailed the first shipments of serum, in quantities sufficient for approximately 6 months, to eight laboratories on January 20, 1958. The distribution of the serum is a continuing function of the Venereal Disease Research Laboratory, but only the results of testing for the 2½-year period January 1958–June 1960 are included in this report, since a single lot of

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serum was used by all participating laboratories during that period. Since June 1960 a volume of serum sufficient for international distribution by WHO and for national distribution by the Venereal Disease Research Laboratory has been prepared and is being made available to laboratories by these organizations in the liquid state.

Control Serum

The control serum, lot No. 1–58, was a pool of serums from 22 syphilitic donors. Each serum was reactive to the TPI test. Serum was sterilized by passage through a Seitz filter pad, bottled in screw-capped vials, and stored at −20° C. until shipped. Instructions accompanied each vial (see insert next column).

TPI Test Techniques

The techniques used in the participating laboratories are described below. Names of these laboratories will be supplied upon request to the Venereal Disease Research Laboratory.

Laboratory 1, Venereal Disease Research Laboratory. The test used is described in detail in the Manual of Serologic Tests for Syphilis. Two hundred 50 percent hemolytic units of complement, rather than a measured amount of guinea pig serum, are used in this test.

Laboratory 2. Technique described in the Manual of Serologic Tests for Syphilis, with the following exceptions:

- Lyophilized complement is used. On day of use it is passed through a Millipore filter to insure sterility.
- Rabbits are killed by intravenous injection of air; they are not exsanguinated.
- Treponemes are no longer extracted on a rocker in a water bath. Instead, a magnetic stirrer, a teflon coated magnetic bar, is used in the air incubator at 33.5° to 34° C.
- Twelve to fifteen treponemes per HPF (high dry field) are used.
- Penicillinase is used routinely in all tests.
- Tests are run in incubator at 33.5° to 34° C. for 20 hours.
- Bacterially contaminated serums are not read. These serums are not filtered and used, since there is no available evidence of the effect of the bacteria on antibodies.

TPI TEST CONTROL SERUM
(Lot No. 1–58)

This Serum Is Sterile

1. Aseptically prepare a 1:10 dilution of this serum with sterile 0.85 percent saline.
2. Into appropriate tubes, pipette 1.0 ml. aliquots of the 1:10 dilution of serum, stopper tightly to avoid dehydration, and place in deep freeze.
3. On first testing day of the week, remove one aliquot of the 1:10 dilution, thaw, mix thoroughly, and heat at 56° C. for 30 minutes.
4. Into tubes numbered 1–4, pipette:

Tube No.	*1:10 dilution of serum (ml.)*	*0.85 percent saline (ml.)*	*Control serum dilution*
1	0.3	0.3	1:20
2	.2	.6	1:40
3	.1	.7	1:80
4	.1	1.5	1:160

5. Mix thoroughly and store in the refrigerator, 6°–8° C.
6. These dilutions are used for 1 working week without additional heating.
7. This range of serum dilutions was selected because the calculated 50 percent immobilization end point obtained with the TPI test technique employed at the Venereal Disease Research Laboratory approximates the dilution of 1 : 70.

- Sensitized sheep cells in test for residual complement: 0.2 ml. of a mixture consisting of 0.1 ml. 1 : 1,000 hemolysis dilution and 0.5 ml. of 5 percent sheep cells.

Laboratory 3. Technique of Nelson and Diesendruck (*2*).

Laboratory 4. Serums are placed in 56° C. water bath for 50 minutes. Penicillinase is then added in proportions of 0.1 ml. to 1.0 ml. of serums. Dehydrated complement is used. To each milliliter of reconstituted complement 0.1 ml. of dihydrostreptomycin is added. On the second day of orchitis and each day thereafter rabbits receive 1 ml. of cortisone intramuscularly. Fourteen or fifteen days after inoculation rabbits are killed by air embolism instead of by exsanguination.

Reading and reporting: A total of 25 treponemes are counted and the percentage of motile organisms is noted. Control tubes on patients should have 70 percent or more motile organisms; tubes 2 and 3, 70 percent or more.

Positive report with active complement, 12 percent or less motile organisms. Doubtful with active complement, 13 to 70 percent motile organisms. When the report is "doubtful," another section of the slide is read so that the percentile is based on a sample of 50 treponemes.

Laboratory 5. Technique described in Manual of Serologic Tests for Syphilis, with two exceptions:

- This laboratory has not yet converted to the use of 200 units of complement.
- The following modifications have been made in reporting: Reactive, control tube (CT), 60 percent; weekly reactive, CT 30–59 percent; inconclusive, CT 18–29 percent; nonreactive, CT 0–17 percent; anticomplementary, 25 percent hemolysis on residual C test; and unsatisfactory, 70 percent mobility in C tube. All specimens reacting in the 18–59 percent range are tested at least twice, and the report is based on the consensus result. All inconclusive reports are accompanied by a consultation requesting a second specimen.

All specimens showing a marked loss of motility in the control tubes are retested with penicillinase. If loss of motility persists after penicillinase, specimens are reported unsatisfactory.

Laboratory 6. Techniques described in Manual of Serologic Tests for Syphilis, with one exception: titration of complement is omitted.

Laboratory 7. Technique of Magnuson and Thompson (*7*), using 22.2 percent complement. Interpretation of results: As described by Nelson and Mayer (*1*).

Procedure for obtaining *T. pallidum* from rabbit testes for transfer inoculation and for use in testing serum: The testes used are 6 to 10 days post inoculation and firm and free of infection, masses, or degeneration.

For transfer inoculation the testes are freed from fascia and fat and are washed three times with sterile normal saline. They are then minced coarsely with scissors, suspended in a solution of 50 percent inactive normal rabbit serum and 50 percent normal saline, centrifuged briefly, filtered, and then inoculated within 10 minutes with 0.25 to 0.5 cc. per testis, depending on the number of *T. pallidum* present.

For use in test the testes are freed, washed, scissors-minced, and placed in survival media. The flask is then shaken in a water bath at 37° C. for 1 to 2 hours, depending on the number of *T. pallidum* present. The solution is then diluted with sterile normal saline to contain from 10 to 12 *T. pallidum* per field, when the readings of five dark fields are averaged.

Laboratory 8. Technique described in Manual of Serologic Tests for Syphilis.

Laboratory 9. Technique of Nelson and Mayer (*1*).

Discussion

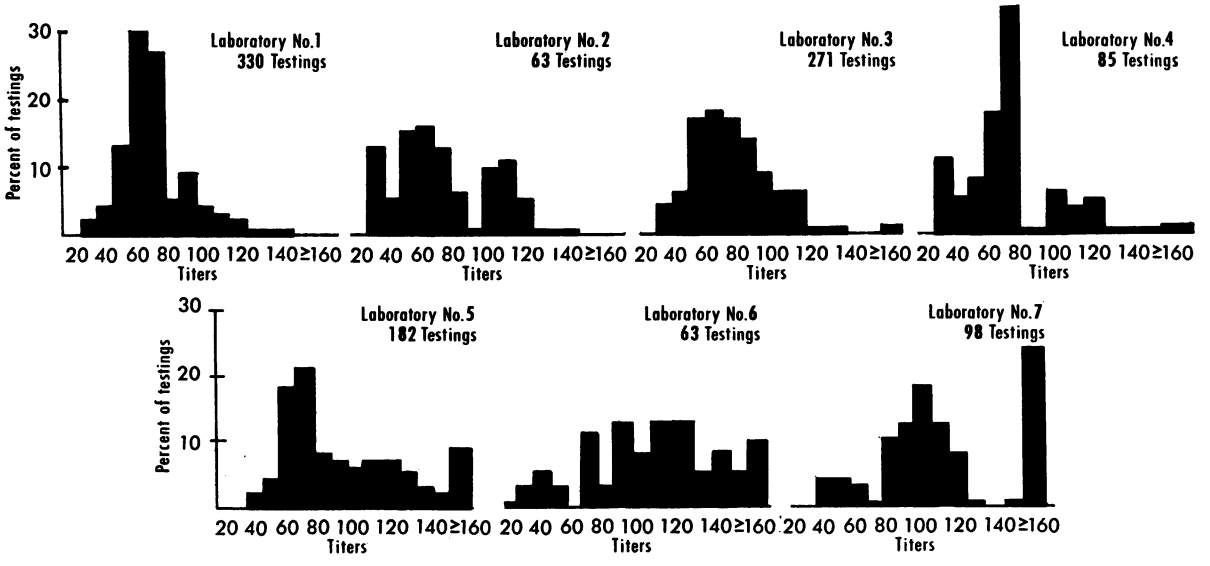
The testing procedures used by the laboratories whose use of a standard control serum is described in this report are not identical and contain many modifications of or additions to the TPI test originally described by Nelson and

Results of use of TPI test control serum, lot No. 1–58, in nine laboratories, January 1958–June 1960

Laboratory No.	January–June 1958		July–December 1958		January–June 1959		July–December 1959		January–June 1960		Total	
	Testings	Average titer	Testings	Average titer	Testings	Average titer	Testings	Average titer	Testings	Average titer	Testings	Average titer
1	75	1:70	78	1:75	74	1:77	50	1:71	53	1:67	330	1:72
2	15	1:68	-------	-------	16	1:74	18	1:82	14	1:73	63	1:75
3	61	1:96	59	1:85	50	1:53	40	1:63	61	1:71	271	1:75
4	6	1:133	16	1:77	13	1:74	24	1:67	26	1:66	85	1:74
5	27	1:74	31	1:89	32	1:77	38	1:72	54	1:103	182	1:85
6	12	1:97	15	1:136	13	1:112	9	1:103	14	1:95	63	1:110
7	28	1:90	18	1:91	28	1:106	24	[1] >1:200	-------	-------	98	>1:122
8	-------	-------	-------	-------	-------	-------	6	1:114	13	1:139	19	1:131
9	5	1:134	-------	-------	-------	-------	-------	-------	-------	-------	5	1:134

[1] In some tests when reactivity end points were not reached, the results were reported as "greater than 1:200."

Results of TPI tests in seven laboratories using control serum from lot No. 1–58 supplied by Venereal Disease Research Laboratory



associates (*1,2*). Other variances in animal or laboratory processing that may have been considered too trivial to report may also exist.

Cumulatively, all elements of a testing procedure that may be controlled, plus those that are uncontrollable and variable, such as the rate and character of treponemal growth in rabbit testes, determine the specificity and sensitivity as well as the reproducibility of a serologic test. Since the TPI test is usually performed on serums from patients who do not have definite clinical diagnoses of syphilis, the relative efficiency of this test each time it is performed is determined principally by the behavior of the test "controls." However, even though these controls indicate that the test is valid, only the titer of a reactive serum defines the relative sensitivity of the test.

Each TPI testing laboratory could prepare and maintain a reactive control serum and through its use could ascertain the daily variance in TPI test sensitivity. When several laboratories use a common quantitatively titered serum for this purpose each laboratory may be informed not only of daily variances but also of the mean reactivity levels of each laboratory's test. In order to provide this type of intra- and extra-mural reference control data, the Venereal Disease Research Laboratory established the service of producing and distributing control serum and receiving, duplicating, and distributing reports periodically to the laboratories desiring to participate in this service. Most of the participating laboratories have expressed a desire to have the service continued.

The table shows the variation in calculated mean titers during each of five 6-month periods. In most of the laboratories variations between testing periods were not large but laboratories 1–5 had lower average test sensitivity levels than did laboratories 6–9. These averages do not show the spread of titers obtained.

As shown in the chart, the frequency distribution of titers is not similar in all laboratories. The findings of laboratories 8 and 9 are not included in the chart because the numbers of testings were too small. All test titers shown for laboratory No. 1, the Venereal Disease Research Laboratory, were between 1 : 35 and 1 : 140 because of a decision to accept only this range of control serum reactivity (± 1 dilution from 1 : 70 dil) as an indicator of a valid test. In the few instances in which higher or lower control serum titers were obtained, the tests were declared invalid and were re-run. The decision to re-run such tests was based on previous experience (*8*) that had indicated that a TPI test set in the range of reactivity presently being used produced fewer probable "false positive" reactions than when it was performed at a more reactive level.

It is now possible for TPI testing laborato-

ries in any country to obtain aliquots of a common control serum from the World Health Organization. The lot of serum now being distributed in the United States by the Venereal Disease Research Laboratory has been divided and placed with WHO for distribution. This serum is an important type of laboratory control for the TPI test and it offers the only means available for continuation and rapid surveillance of the level of reactivity of the TPI tests that are being performed in many laboratories and in many countries.

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