Treponemal Tests in Diagnosis of Syphilis and Biologic False Positive Reactors

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THE ERA of the treponemal tests began in 1949 with the introduction of the *Treponema pallidum* immobilization (TPI) test by Nelson and Mayer (*1*). The TPI test was followed in 1955 by the *T. pallidum* complement fixation (TPCF) test, developed by the Public Health Service (*2*).

The TPCF test has been almost entirely replaced by the Reiter protein complement fixation (RPCF) test established in 1957 (*3–5*). This test employs the protein fraction of the nonpathogenic strain of *T. pallidum*, originally described by D'Alessandro and his co-workers in 1949 (*6,7*). Since the Kolmer technique is used in the RPCF test, the procedure is also referred to as the Kolmer-Reiter protein complement fixation test (KRPCF). A new treponemal test, the fluorescein tagged antibody (FTA) test (*8*) is still under study and is not available for general use.

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The RPCF and the TPCF tests for this study were performed under the supervision of Genevieve O. Stuart, chief serologist, State Wassermann laboratory. The TPI tests were performed under the direction of Dr. Irving H. Blank, department of dermatology and syphilology, Massachusetts General Hospital, Boston.

There are three uses for treponemal tests: (*a*) to help distinguish between biologic false positive and true positive blood tests for syphilis; (*b*) to help establish a diagnosis of syphilis in patients who have clinical evidence of the disease, particularly evidence of late syphilis, but who have negative blood and spinal fluid serologic tests; and (*c*) to assist in the diagnosis of syphilis in patients with epidemiological evidence of the disease but with negative clinical and serologic findings (*9,10*).

The division of venereal diseases of the Massachusetts Department of Public Health performs both the TPI and RPCF tests at the request of private physicians and hospitals. The TPI test has been performed for patients of private physicians since 1955. The TPCF test was added in 1956 but was replaced by the RPCF test in 1958. Performance of these tests has given us an opportunity, as never before, to evaluate the incidence and prevalence of biologic false positive reactors in Massachusetts.

Basic Medical Data

Physicians and hospitals who request a TPI or RPCF test are asked to complete a form which summarizes the results of the diagnostic workup to date. The following information is requested.

History

Is there a history of syphilis? If so, how much treatment has the patient received, in what year, and where?

Is there a history of any venereal disease?

Physical Examination

Is there any evidence of primary, secondary, or late symptomatic syphilis or sequelae of these stages?

Are there any stigmata of congenital syphilis?

Diseases and Immunizations Causing False Positive Reactions

Is there any evidence of a disease commonly causing a biologic false positive reaction? (A list is given on the form.)

Epidemiological Investigation

Give the result of your examination, including the blood test for syphilis on the patient's spouse, parents, children, or siblings, as indicated.

Laboratory Tests

Record the results of at least two blood tests for syphilis.

Give the date of the lumbar puncture and the result of your examination of the spinal fluid, including cell count, total protein, and serology.

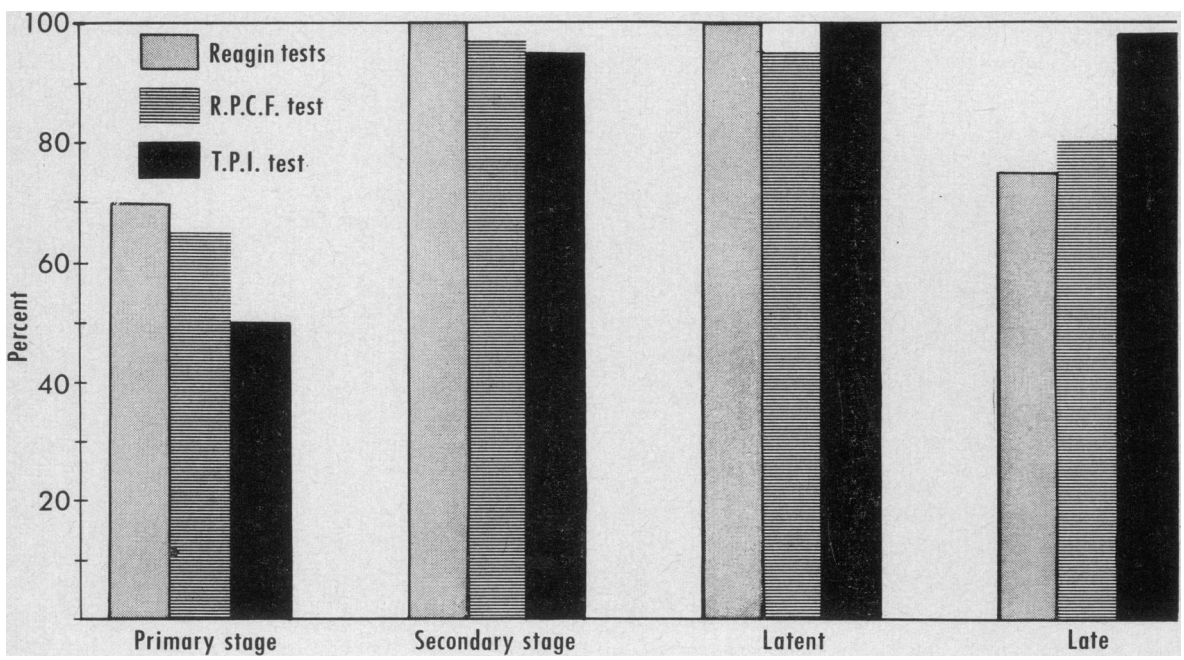
One or more of the above requirements are waived for good and sufficient medical and social reasons. For example, the lumbar puncture is not required in a pregnant patient, a patient with a "bad back," a debilitated patient, or a patient who flatly refuses to have a lumbar puncture done. Examination of the marital partner or other epidemiological investigations are postponed when the patient asks that his family not be tested at this time. No exceptions are made to the requirement that the results of at least two blood tests be reported.

On receipt of the completed summary from the physician or hospital, the division of venereal diseases makes an appointment with the State cooperating venereal disease clinic for the patient to have blood drawn for the TPI test because special techniques are required for this test. The clinic sends a split sample of the patient's serum to the Public Health Service Venereal Disease Research Laboratory at Chamblee, Ga., so that the same blood sample may be tested in two different laboratories. This procedure safeguards against laboratory errors, which are on the negative rather than on the positive side, and imparts greater reliability to the test results. From each blood sample taken for the TPI test, serum is saved for the RPCF test.

To each physician or hospital requesting only the RPCF test, the division of venereal diseases sends a laboratory slip which is to be completed and wrapped around a tube containing 8 cc. of clotted blood. This tube is to be mailed to the Wassermann laboratory.

All laboratories send reports of both the TPI and RPCF tests direct to the division of vene-

Figure 1. Sensitivity of reagin, RPCF, and TPI tests in untreated syphilis



real diseases. If there are no conflicts in the test results, the reports are mailed to the referring physician or hospital.

As experience with these two tests was gained over the years, the policy was adopted of using the RPCF test as a screening device on patients with reactive blood Hinton tests. If the RPCF test was positive, the diagnosis of syphilis could be confirmed, but if this test was negative, a TPI test was advised. Whenever a TPI test was done, the RPCF test was also performed.

Sensitivity and Specificity of Tests

The physician is concerned primarily with determining whether a patient with a positive blood Hinton test does or does not have syphilis. For this reason, he must know the limitations of the treponemal tests. These limitations can best be described by comparing the sensitivity of these tests in the various stages of untreated syphilis with the sensitivity of the older Hinton test.

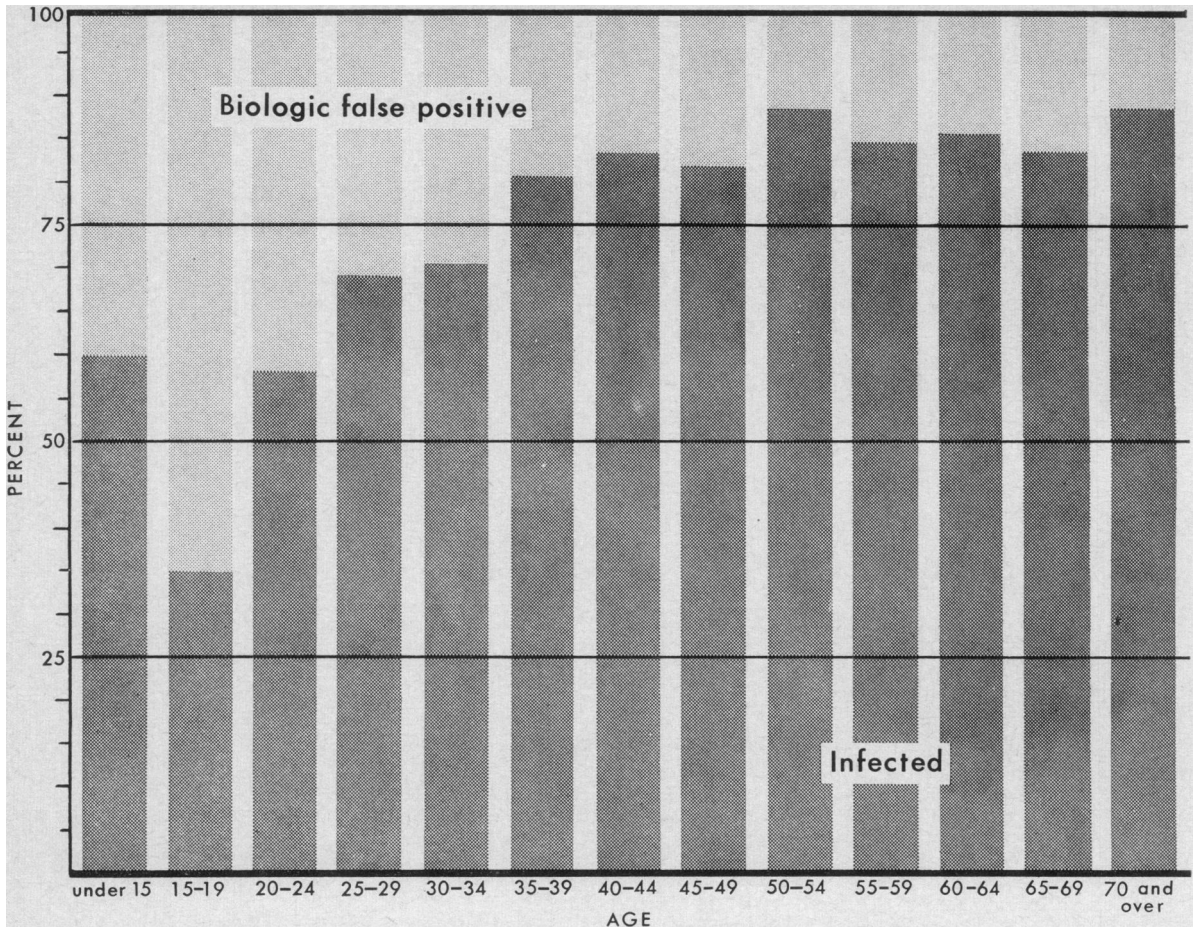
The RPCF test is more sensitive than the TPI test in early syphilis, where we don't particularly need a high degree of sensitivity, and less sensitive in latent and late syphilis, where we do want and need a more sensitive test (fig. 1). Thus, in primary syphilis, while the Hinton test is positive in 70 percent of the cases, the RPCF test has a sensitivity of 65 percent, the TPI test only 50 percent. In secondary syphilis, the Hinton test is always positive, but the RPCF test is positive in only 98 percent of cases, and the TPI test in 95 percent. In latent syphilis, the Hinton test is of necessity positive in all cases. The TPI test is practically always positive, too, but the RPCF test is positive in only 95 percent of the cases. In late syphilis, the Hinton test is positive in 75 percent of the cases, the RPCF test in 85 percent, and the TPI in 98 percent.

Although absolute percentages have been given to depict the level of sensitivity of these tests in the various stages of untreated syphilis, these percentages indicate only the average range of sensitivity. Any or all tests may vary from their assigned sensitivities in a given case and in accordance with the test technique used. Thus, in patients with a syphilitic chancre of 1 day's duration, the blood test will be positive in only about 25 percent. At the beginning of the second week after infection, approximately 50 percent of such patients become seropositive. The number increases to 75 percent at the beginning of the third week and to 100 percent by the fourth week, by which time most patients exhibit signs and symptoms of secondary syphilis. Similarly, a patient with late secondary syphilis has a greater chance of having positive RPCF and TPI tests than one in the early secondary stage. Results of the treponemal test may not show the same level of sensitivity when different techniques are used or when different laboratories perform these tests, even on the same blood sample.

Table 1. Syphilitic infection in 703 patients with positive reactions to the Hinton test, Massachusetts, 1954–59

Classification	Total reactors	Infected	
		Number	Percent
Total	703	548	78.0
Clinic or private:			
Clinic	548	451	82.3
White	332	243	73.2
Nonwhite	216	208	96.3
Private	155	97	62.6
White	146	88	60.3
Nonwhite	9	9	100.0
Race:			
White	478	331	69.2
Male	231	176	76.2
Female	247	155	62.8
Nonwhite	225	217	96.4
Male	79	74	93.7
Female	146	143	97.9
Sex:			
Male	310	250	80.6
Female	393	298	75.8
Marital status:			
Single	169	114	67.5
Male	91	68	74.7
Female	78	46	59.0
Married	365	283	77.5
Male	172	139	80.8
Female	193	144	74.6
Widowed	78	66	84.6
Male	22	19	86.4
Female	56	47	83.9
Separated	53	51	96.2
Male	16	15	93.8
Female	37	36	97.3
Divorced	38	34	89.5
Male	9	9	100.0
Female	29	25	86.2

Figure 2. Results of treponemal tests of 703 patients, by age, Massachusetts, 1954–59



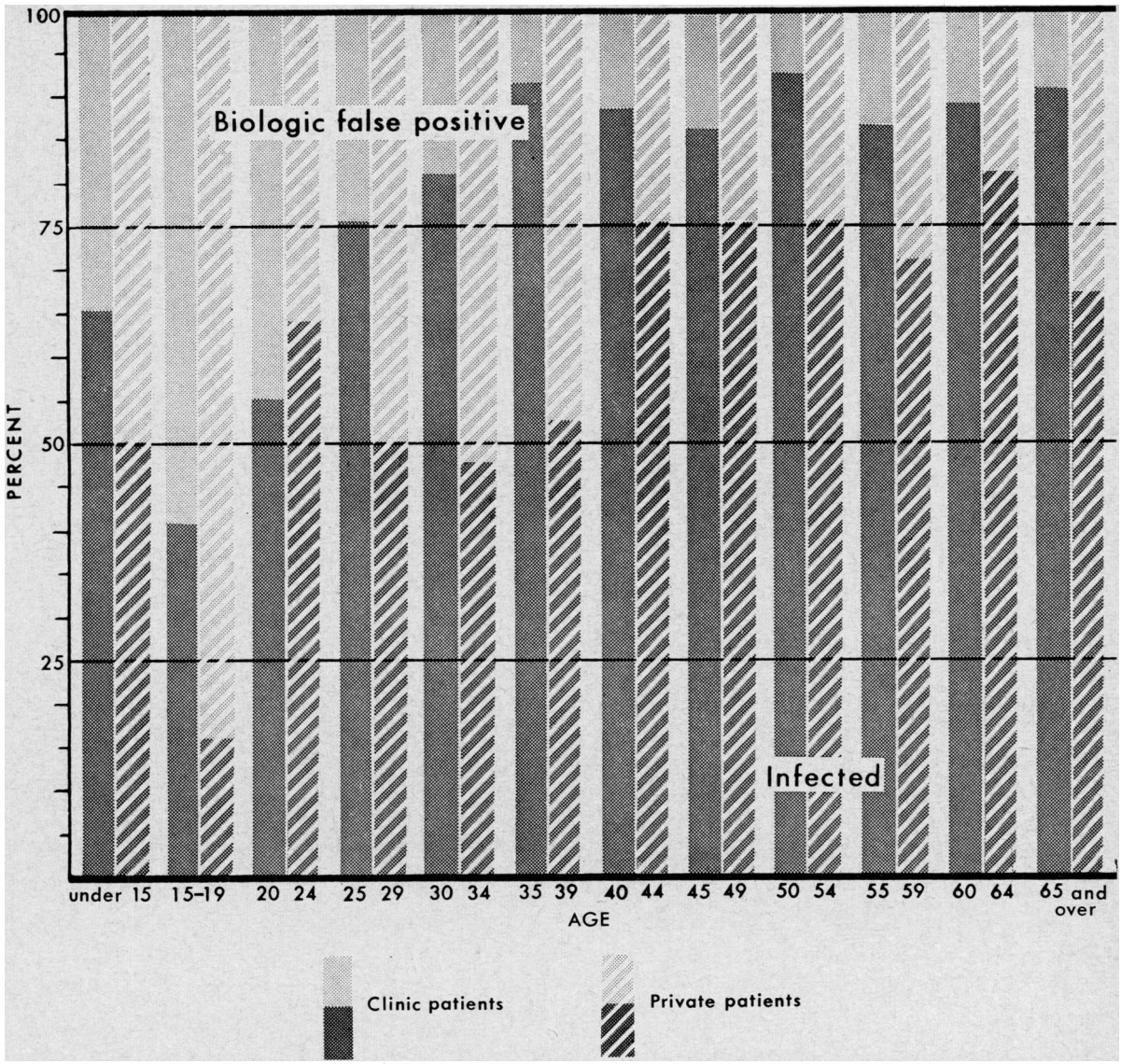
At the present stage of knowledge of and experience with the treponemal tests, a positive reaction, for all practical purposes, means syphilis or a treponemal disease, namely, yaws, pinta, or bejel.

Types of Cases Studied

This report covers an analysis of 703 positive Hinton reactors who, on the basis of the study forms submitted by their physicians had no historical, physical, or epidemiological evidence of syphilis. Excluded are (*a*) patients who had clinical evidence of syphilis but who had either positive or negative blood and spinal fluid serologic tests, (*b*) patients who had epidemiological evidence of syphilis but had negative clinical and serologic findings, (*c*) patients who had had not only a positive blood Hinton test but also positive spinal fluid serology (a positive spinal fluid serology means neurosyphilis) and whose TPI or RPCF test was positive too, and (*d*) patients who had an initial positive qualitative Hinton test but whose later quantitative Hinton tests were negative on at least two occasions. Thus, the study group is composed entirely of patients with persistent positive blood Hinton tests whose physicians could not decide whether or not they had syphilis.

Of the 703 cases studied, 155 (22 percent) were patients of private physicians and 548 (78 percent) were from clinics and hospitals. There were 478 (68 percent) white patients and 225 (32 percent) nonwhite. There were 310 (44.1 percent) males, and of these, 231 (74.5 percent) were white and 79 (25.5 percent) nonwhite. Females numbered 393 (55.9 percent), and of these 247 (62.9 percent) were white and 146 (37.1 percent) nonwhite. As regards marital status, 169 patients (24 percent) were single, 365 (51.9 percent) were married, 78 (11.1

Figure 3. Results of treponemal tests of 703 clinic and private patients, by age, Massachusetts, 1954–59



percent) were widowed, 53 (7.5 percent) were separated, and 38 (5.4 percent) were divorced.

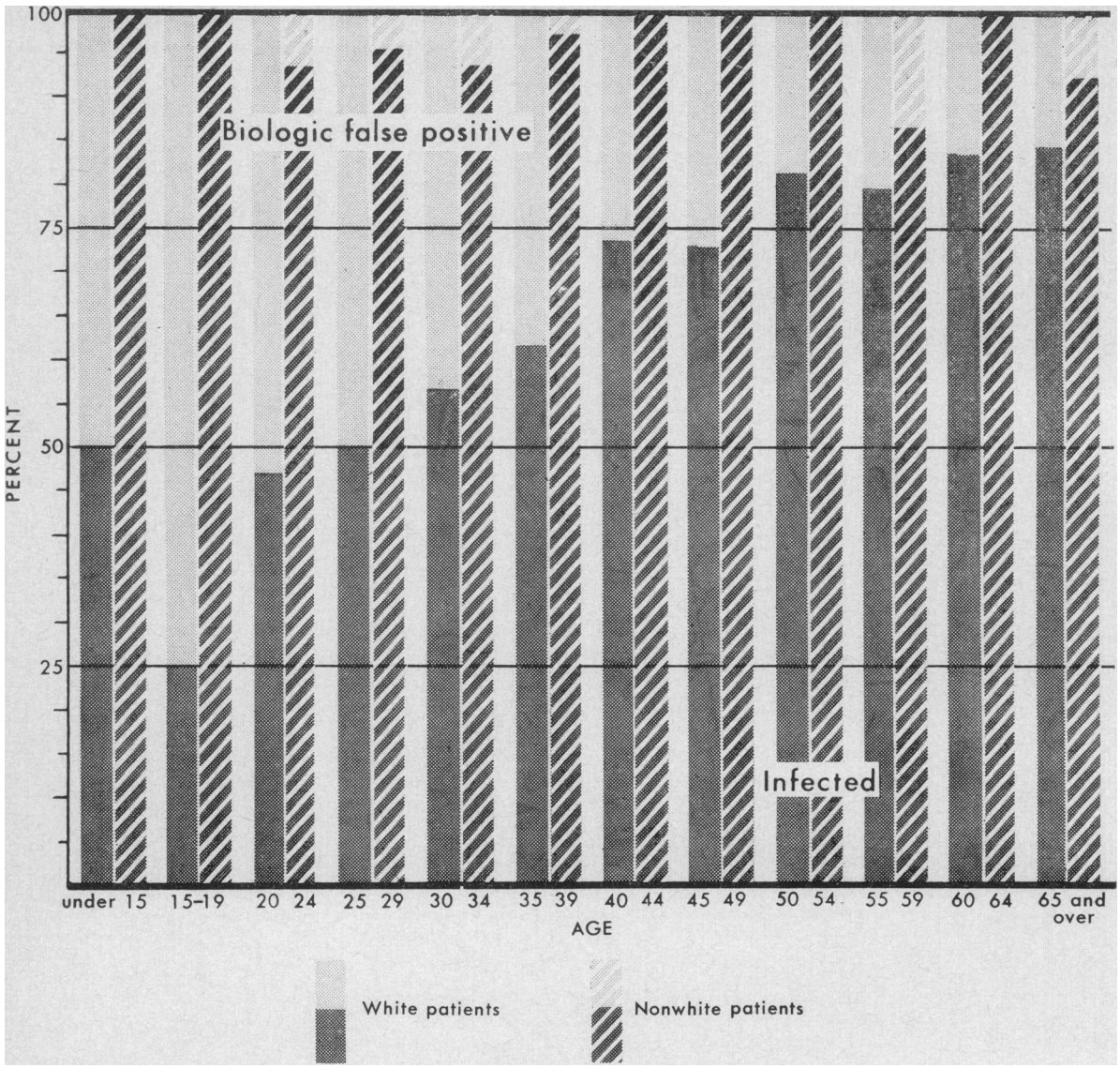
Infection Rates

On the basis of a positive treponemal test, either the TPI or RPCF test, or both, 548 (78 percent) of the 703 patients were infected, whereas 155 (22 percent) were not infected, but had a biologic false positive reaction. The detailed data on infection are given in table 1.

Clinic patients were infected more frequently (82.3 percent) than private patients (63 percent). Nonwhite patients had syphilis more often (96.4 percent) than white patients (69.2 percent) irrespective of whether they were clinic patients or patients of private physicians. Furthermore, the infection rate in nonwhites of both sexes was higher than in white persons. But the infection rate was about the same in males and females of the same race.

When the 703 patients were studied by 5-year age groups, an interesting pattern was seen. With the exception of the group aged 15–19 years, the infection rate increased with age up to about 45 years, when it tended to level

Figure 4. Results of treponemal tests of 703 patients, by race and age, Massachusetts, 1954–59



off (fig. 2). This pattern was seen in both private and clinic patients (fig. 3) and in white patients (fig. 4), but nonwhite patients in this age group failed to show the sharp decrease in infection rate.

The infection rate in both males and females followed the general trend of increasing infection with age, but with a sharp increase in the BFP rate in the 15- to 19-year age group. White males, and to a greater extent, white females, showed this sharp increase in BFP rate in the 15- to 19-year age group, but the infection rate in neither the nonwhite males nor the nonwhite females followed this pattern.

In general, the rate of infection was highest in the larger cities, particularly among patients of private physicians, and to a lesser extent, among clinic patients (table 2). The general policy of the State department of public health is that no patient be "closed out" as a biologic false positive reactor without the benefit of a treponemal test, even though the diagnosis is obvious.

Boston, the largest city in the State, had the highest infection rate (85 percent). Among census tract districts, the infection rate varied from a high of 100 percent to a low of 50 percent. Many of the districts with a

known high prevalence of syphilis also had a higher infection rate among their diagnostic problem cases.

When the data were analyzed according to individual referring hospitals, the infection rate among the 703 diagnostic problem cases generally reflected the prevalence of syphilis in the population group from which the patients came (table 3). In hospitals drawing their patients from areas with a high syphilis prevalence—Boston City Hospital and Massachusetts Memorial Hospital, for example—these diagnostic problem patients showed a higher infection rate than patients in hospitals in areas of lower syphilis prevalence.

Lastly, an attempt was made to correlate the highest dilution positive Hinton test with the diagnosis of biologic false positive reactor. The highest dilution positive titer recorded was 1:16, and this in one case only (fig. 5). In general, BFP reactors have low titer serologic tests, although exceptions to this have been seen by almost all physicians. But among these 155 BFP reactors, only 1 patient had a positive Hinton test at a dilution of 1:16; none in more diluted serum.

Table 2. Results of treponemal tests on 703 positive reactors to the Hinton test, by type of patient and community size, Massachusetts, 1954–59

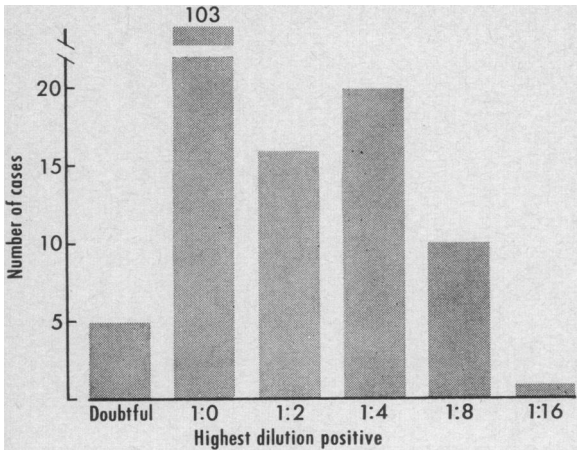
Community size	Cases		Type of patient											
			Private				Clinic				Total			
			Infected		BFP [1]		Infected		BFP [1]		Infected		BFP [1]	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Boston [2]	400	57	31	69	14	31	309	87	46	13	340	85	60	15
100,000–149,999	7	1	2	40	3	60	1	50	1	50	3	43	4	57
50,000–99,999	124	18	28	72	11	28	62	73	23	27	90	73	34	27
All other	172	24	36	55	30	45	79	87	27	13	115	67	57	33
Total	703	100	97	63	58	37	451	82	97	18	548	78	155	22

[1] Biologic false positive.
[2] Population 724.702.

Table 3. Results of treponemal tests on 703 positive reactors to the Hinton test, by source of request, 1954–59

Source of request	Total	Infected		Biologic false positive	
		Number	Percent	Number	Percent
Total	703	548	78.0	155	22.0
Voluntary hospitals	535	440	82.2	95	17.8
Beth Israel	14	9	64.3	5	35.7
Boston City	103	95	92.2	8	7.8
Boston Dispensary	24	18	75.0	6	25.0
Massachusetts General	188	148	78.7	40	21.3
Massachusetts Memorial	138	127	92.0	11	8.0
Peter Bent Bringham	26	17	65.4	9	34.6
All other	42	26	61.9	16	38.1
State hospitals	13	11	84.6	2	15.4
Private physician	155	97	63.0	58	37.0

Figure 5. Highest dilution Hinton positive tests among 155 biologic false positive reactors, Massachusetts, 1954–59



Summary

The division of venereal diseases of the Massachusetts Department of Public Health made a study of 703 patients who had persistently positive blood Hinton tests and who had no historical, physical, or epidemiological evidence of syphilis, on the basis of protocols submitted by their physicians. Each of these patients represented a diagnostic problem to the private or clinic physician, and a treponemal test was indicated.

When the *Treponemal pallidum* immobilization (TPI) and the Reiter protein complement fixation (RPCF) tests were positive, the diagnosis of syphilis could be confirmed. When an RPCF test was negative, the physician was advised to have his patient have a TPI test. If both tests were negative, the patient could be classified as a biologic false positive (BFP) reactor, with a few reservations. The sensitivities of the TPI and the RPCF tests were compared with the sensitivity of the Hinton tests in the various stages of untreated syphilis.

On the basis of all available data, including the treponemal tests, 548, or 78 percent, of the 703 diagnostic problem patients were found to be infected, and 155 (22 percent) were BFP reactors. Approximately 70 percent of the white and 96 percent of the nonwhite patients had syphilis. The infection rate was about 82 percent in clinic patients and 63 percent in private patients. More cases of syphilis were discovered in both men and women who were married or who had been married than in single persons.

In these diagnostic problem cases, with the exception of white patients aged 15 to 19 years, the number of cases of syphilis discovered increased up to age 45, when the infection rate tended to level off. Of practical interest was the sharp increase in BFP reactions in white patients in the age group 15–19 years.

More syphilis was found in the larger cities, and the highest rate was in Boston, the largest city in the study area. Areas in Boston and elsewhere in the State which have a higher syphilis prevalence also showed a higher infection rate among these diagnostic problem cases. This was reflected in the number of syphilis cases in the hospitals drawing their patients from these areas.

When the 155 patients with BFP reactions were studied to correlate their highest dilution serologic titers with their diagnoses, it was found that only one had a positive dilution titer of 1:16; all others had lower positive titers. This coincides with the experience of physicians that, generally speaking, patients with BFP reactions have low-titer serologic titers. Many exceptions can be found, however.

Results of this study indicate strongly that the reagin tests are still valuable in the diagnosis of syphilis. Even when diagnostic problems arise, almost 80 percent of patients with a persistently positive blood reagin test have or have had syphilis. For this reason, such patients must be considered to be syphilitic until proved otherwise. Today, a diagnosis of BFP should not be made in Massachusetts without the benefit of the RPCF test as a screening device, and if this test is negative, the TPI test should be performed. Only when both treponemal tests are negative can the diagnosis of biologic false postive reaction be entertained in a patient with a persistently positive reagin blood test.

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Training Courses

Mobilization. Four courses to train medical and health personnel in emergency civil defense services are scheduled for fiscal year 1961 by the Public Health Service and the Office of Civil and Defense Mobilization. Three of them, offered for the first time, are for hospital administrators, registered nurses, and environmental health personnel. The fourth repeats basic health mobilization training for physicians and health-related professions given in the spring of 1960. All courses cover basic civil defense concepts, current information on biological, chemical, and radiological warfare, and community disaster planning. Tuition and housing are without cost to students and about half of necessary travel expenses are reimbursable through OCDM student training funds. Enrollments are limited. Apply through State civil defense directors. The courses are:

Health Mobilization Program for Emergency Hospital Management. OCDM Eastern Instructor Training Center, Brooklyn, N.Y., December 4–9, 1960. (Professional endorsement of the American Hospital Association.)

Nursing Aspects of Health Mobilization. OCDM Staff College, Battle Creek, Mich., April 23–28, 1961.

Environmental Health Aspects of Health Mobilization. Battle Creek, Mich., April 23–28, 1961.

Health Services Aspects of Health Mobilization. OCDM Eastern Instructor Training Center, Brooklyn, N.Y., May 7–12, 1961.

Sanitary Engineering. Training courses scheduled by the Robert A. Taft Sanitary Engineering Center include a course on medical and biological aspects of air pollution for physicians, veterinarians, and control officials dealing with health-related hazards, and another on determining antibiotic and pesticide residues in milk for professional people in regulatory and control agencies and in industry.

Medical and Biological Aspects of Air Pollution. Primarily treats health aspects of air pollution as observed in recorded incidents and explored through laboratory and epidemiological investigations. Also outlines concepts of an engineering program for supplementing assessment of a community's air pollution problem. December 12–16, 1960.

Determination of Antibiotic and Pesticide Residues in Milk. Detection techniques and procedures, including sessions for exchange of problems encountered by responsible officials. December 12–16, 1960.

Direct requests for more information and applications to the Chief, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio, or to a Public Health Service regional office director.