Immune-Adherence Test for Syphilis

—Comparison With TPI and VDRL Slide Tests—

By SIDNEY OLANSKY, M.D., AD HARRIS, and HELEN CASEY, M.S.

Application of the adhesion phenomenon to detection of treponemal antibodies, if found practicable, may provide valuable assistance in syphilis diagnosis. Of particular practical significance is the use of killed T. pallidum *suspensions in the test.*

A REACTION between human erythrocytes and treponemes sensitized by antibody from syphilitic serum was first described by R. A. Nelson, Jr. (*1*, *2*). In a later report (*3*) it was stated that "The specificity of this reaction for antibody in syphilis serum was established by examination of three hundred eighty-five (385) human sera." This report also stated that the results of this test, referred to as the immune-adherence phenomenon or reaction, "corresponded with the clinical diagnosis of syphilis and with the results of the TPI test [*Treponema pallidum* immobilization] in all except five (5) instances."

Since the immune-adherence reaction is technically a much simpler procedure than the TPI test and therefore would be a desirable substitute if found to be equally efficient, a study to compare these tests, not only with each other, but with the VDRL slide test and clinical diagnoses, was begun. The purpose of this report is to present findings obtained with these three serologic tests for syphilis and to compare these results with diagnoses and medical opinion. The immune-adherence reaction will be herein referred to as the IA test.

Dr. Olansky is director and Mr. Harris is associate director, and also in charge of the Serology Section, Venereal Disease Research Laboratory, Public Health Service, Chamblee, Ga. Mrs. Casey is a bacteriologist at the laboratory.

Methods

IA Test

Treponeme suspension was prepared from testicular syphilomas of rabbits. Mixtures containing 0.6 ml. of treponeme suspension, 0.1 ml. guinea pig serum (complement), 0.1 ml. of serum, and 0.2 ml. of washed human erythrocytes were heated at 37° C. for 30 minutes. The tubes containing these mixtures were then centrifuged (500 r.p.m., 5 min.) and the treponemes in the fluid phase were counted by dark-field microscopy. Controls including combinations of known positive and negative serums, with and without complement, and guinea pig

serum in saline were always a part of each test run.

The treponeme suspension used was adjusted so that approximately 100 organisms were present in 10 fields in the negative serum and in complement controls. Each test was read by placing 0.01 ml. of the fluid phase under a 22 mm.^{2} cover slip and counting the number of treponemes observed in 10 fields, using the high-dry objective of a darkfield microscope.

TPI Test

The method of Nelson and Mayer (*4*, *5*) as modified in this laboratory (*6*) was used in this study. Modifications included a five times increase of thioglycollate in the survival medium and a fourfold increase of complement.

VDRL Slide Test

The technique used for the VDRL slide test was exactly as originally reported (*7*, *8*) using antigen prepared and standardized at the Venereal Disease Research Laboratory.

Adequate Therapy. The syphilis patients in this study were considered to have had adequate therapy if they had received either: (*a*) 2.4 million units of penicillin or more, or, (*b*) 20 or more injections of arsenicals plus 20 or more injections of heavy metals. Good evidence regarding the syphilis status of the 305 patients selected for this study was obtained from sources other than the Venereal Disease Research Laboratory. These data included the results of physical examinations, personal and family histories, and laboratory findings. The results of laboratory tests recorded in this pa-

Table 1. Reactivity of the IA test on 234 syphilitic and 71 presumably nonsyphilitic patients

Zones	Treponeme count	Syphilitic patients (all stages)		Presumably nonsyphilitic patients	
		Number	*Percent*	*Number*	*Percent*
A	0–10	126	54	6	8.4
B	11–20	32	24	12	36.6
	21–30	25		14	
C	31–40	15	22	10	55.0
	41–50	10		6	
	51–60	7		5	
	61–70	6		6	
	71–80	5		4	
	Over 80	8		8	
Total		234	100	71	100

Table 2. Effects of time since adequate therapy or of absence of adequate treatment on the IA test performed on 234 syphilitic patients

Stage of syphilis, treatment received, and time since adequate treatment	IA test (treponeme count zones)			
	A	B	C	Total
Primary (44 patients)				
Untreated	5	3	1	9
Inadequately treated	1	----	----	1
Time since adequate treatment:				
0–6 months	7	5	----	12
6–12 months	2	1	----	3
1–2 years	----	----	3	3
3–5 years	2	1	9	12
6–10 years	1	1	1	3
Over 10 years	1	----	----	1
Total	19	11	14	44
Secondary (54 patients)				
Untreated	7	1	----	8
Inadequately treated	1	----	----	1
Time since adequate treatment:				
0–6 months	5	3	----	8
6–12 months	1	4	----	5
1–2 years	3	2	3	8
3–5 years	4	9	9	22
6–10 years	1	----	1	2
Over 10 years	----	----	----	----
Total	22	19	13	54
Latent (74 patients)				
Untreated	17	11	9	37
Inadequately treated	6	4	9	19
Time since adequate treatment:				
0–6 months	14	----	----	14
6–12 months	----	----	----	----
1–2 years	2	----	----	2
3–5 years	----	----	----	----
6–10 years	----	----	----	----
Over 10 years	1	1	----	2
Total	41	16	18	74
Late (62 patients)				
Untreated	27	7	4	38
Inadequately treated	14	2	2	18
Time since adequate treatment:				
0–6 months	4	----	----	4
6–12 months	----	----	----	----
1–2 years	----	----	1	1
3–5 years	----	1	----	1
6–10 years	----	----	----	----
Over 10 years	----	----	----	----
Total	45	10	7	62

per did not influence medical opinion, since these tests were performed after diagnoses were made.

Results

Table 1 shows the results of the IA test, in count groupings of 10, obtained on the 234 syphilitic and 71 presumably nonsyphilitic patients. Since positive, doubtful, and negative count zones have not been established for the IA test, 3 zones of reactivity have been selected for this discussion of the results obtained with this procedure. These zones are designed as A (0–10 treponemes, inclusive), B (11–30 treponemes, inclusive), and C (all treponeme counts greater than 30). The greatest degree of reactivity among the syphilitic patients is represented by the A zone since the reaction of the tested serum is maximum, or nearly so, when the treponeme count of the supernate is reduced to 10 or fewer. The B zone represents an intermediate type of reaction in which 11 to 30 of the treponemes are apparently unaffected by the serum being tested. The least reaction is encountered in the C zone, where 31 or more of the treponemes remain in the supernate.

More than half of the IA test results on syphilitic donors were in the A zone, while a similar percentage of findings were in the C zone on presumably nonsyphilitic donors. It therefore appears that syphilis, past or present, may influence the IA test. However, since all of the serums from syphilitic donors did not produce maximum reactions, and since some of these patients had had various amounts of treatment at some time prior to testing, a listing of results as related to therapy and time periods was made. This information is shown in table 2.

The results recorded in table 2 show that IA test findings may fall into all 3 groupings (A, B, and C) in each of 4 categories of syphilis listed. A greater number of these findings are in the A and A plus B groupings than were found in the C zone. Although 25 of these patients who had received either no specific therapy or inadequate treatment gave C-zone reactions, only one of these was found in the primary, and none in the secondary syphilis categories. Identical numbers of C-zone reactions were obtained from the primary and secondary syphilis patients who had had adequate therapy one or more years prior to testing date. The findings indicate that early syphilis, untreated, inadequately treated, or adequately treated less than 1 year before testing date, may produce an A- or B-zone finding in the IA test.

In the test results obtained on patients having latent or late syphilis, a slightly different picture is seen. A larger proportion of the test results in these clinical categories fall into the C zone, even though adequate therapy had not been received. The number of patients in each post-therapy observation period is not great enough to indicate the effect of treatment, in late or latent syphilis, on IA test findings.

The TPI and VDRL slide tests, in addition to the IA test, were performed on serums from each of the patients in this study in order to ascertain the relative position of these tests as aids in diagnosis. In no instance were the test results reported in this paper used for determining the status of syphilitic or presumably

Table 3. Relative reactivities of the IA, TPI, and VDRL slide tests for syphilis on 44 patients with primary syphilis diagnoses (treated or untreated)

IA test		TPI test			VDRL slide test		
Zone	Number of patients	Positive	Doubtful	Negative	Positive	Weakly Positive	Negative
A (0–10 treponemes)	19	11	0	8	9	1	9
B (11–30 treponemes)	12	7	0	5	1	2	9
C (31 or more treponemes)	13	0	0	13	1	0	12
Total	44	18	0	26	11	3	30

Table 4. Relative reactivities of the IA, TPI, and VDRL slide tests for syphilis on 54 patients with secondary syphilis diagnoses (treated and untreated)

IA test		TPI test				VDRL slide test		
Zone	Number of patients	Positive	Doubtful	Negative	Inconclusive	Positive	Weakly positive	Negative
A (0–10 treponemes)	22	15	1	5	1	10	1	11
B (11–30 treponemes)	19	6	0	10	3	4	2	13
C (31 or more treponemes)	13	3	0	10	0	0	1	12
Total	54	24	1	25	4	14	4	36

nonsyphilitic donors. The relative reactivities of these three tests are shown in tables 3–6. These tables are constructed so that agreement and total reactivity of each test may be visualized.

Table 3 shows that 19 A-zone reactions with the IA test, 18 positive reactions with the TPI test, and 14 positive or weakly positive reactions with the VDRL slide test were obtained on the 44 patients with primary syphilis (treated or untreated). The agreement between these tests appears to be closer when presented in this manner than it actually is, since the 19 zone A reactions in the IA test were actually accompanied by only 11 positive reactions in the TPI test and 10 positive or weakly positive reactions in the VDRL slide test. Seven of the 12 zone B reactions in the IA test were accompanied by positive reactions in the TPI test and only 3 of these gave positive or weakly positive reactions in the VDRL slide test. In this group more positive reactions were obtained with the IA and TPI tests than with the VDRL slide test. This may be a reflection of treatment effect since only 10 of the 44 patients in this group had not had adequate treatment.

In the secondary syphilis group, table 4, the greatest number of positive reactions were obtained with the TPI and IA tests and only 9 of these 54 patients had not had adequate treatment. The results listed in both tables 3 and 4 show that a similarity exists between the TPI and IA tests in that positive reactions may be obtained by these tests, in adequately treated early syphilis, after the VDRL slide test has returned to negativity.

As is shown in tables 5 and 6, there is closer agreement between the three tests in late or latent syphilis (treated or untreated) than was obtained in the early syphilis groups, under the same circumstances. Forty A-zone reactions with the IA test were accompanied by 36 positive TPI and 36 positive or weakly positive VDRL slide test results in the latent syphilis

Table 5. Relative reactivities of the IA, TPI, and VDRL slide tests for syphilis on 74 patients with latent syphilis diagnoses (treated and untreated)

IA test		TPI test				VDRL slide test		
Zone	Number of patients	Positive	Doubtful	Negative	Inconclusive	Positive	Weakly positive	Negative
A (0–10 treponemes)	40	36	0	1	3	32	4	4
B (11–30 treponemes)	16	3	0	13	0	5	1	10
C (31 or more treponemes)	18	13	1	4	0	2	0	16
Total	74	52	1	18	3	39	5	30

Table 6. Relative reactivities of the IA, TPI, and VDRL slide tests for syphilis on 62 patients with late syphilis diagnoses (treated and untreated)

IA test		TPI test				VDRL slide test		
Zone	Number of patients	Positive	Doubtful	Negative	Inconclusive	Positive	Weakly positive	Negative
A (0–10 treponemes)	45	42	0	2	1	37	2	6
B (11–30 treponemes)	10	7	0	2	1	2	0	8
C (31 or more treponemes)	7	4	1	2	0	0	2	5
Total	62	53	1	6	2	39	4	19

group. Forty-five A-zone reactions with the IA test, in the late syphilis patients, were accompanied by 42 positive TPI and 39 positive or weakly positive VDRL slide test results. In these two groups (74 latent and 62 late syphilis) only 24 had had adequate therapy, and 18 of these had had this treatment less than 6 months before these tests were made. It is therefore probable that these figures do not show the pattern obtained in early syphilis because, in most instances, enough time had not elapsed after adequate therapy to reverse the positive reaction in the VDRL slide test.

In table 7 the results obtained with the three serologic tests on serum from 71 presumably nonsyphilitic individuals are listed. Several patients in this group were considered to be "biological false positive" reactors because of some degree of reactivity in one or more tests for syphilis, in the absence of signs or symptoms of infection, past or present, and with negative personal and family histories. Had all of these been omitted, on the basis of reactivity in 1 or more of the tests listed in table 6, then 1 or even all 3 of these tests could have been made to appear as being 100-percent specific. The results of the serologic tests herein presented were, in no instance, used as an aid in establishing diagnoses. Attending physicians did, however, have the aid of laboratory findings in making diagnoses.

The presumably nonsyphilitic group is more heavily seeded with positive reactors, in all three tests, than might randomly be encountered. However, this grouping allows the demonstration of what can happen when diagnoses of syphilis are finally resolved on serologic test findings. If it is presumed that a positive reaction in the TPI test is not encountered in the absence of treponemal infection and if, therefore, the 9 serums that react positively in this test are deleted, then the A-zone reac-

Table 7. Relative reactivities of the IA, TPI, and VDRL slide tests for syphilis on 71 presumably nonsyphilitic patients

IA test		TPI test				VDRL slide test		
Zone	Number of patients	Positive	Doubtful	Negative	Inconclusive	Positive	Weakly positive	Negative
A (0–10 treponemes)	6	1	0	4	1	2	0	4
B (11–30 treponemes)	26	2	0	23	1	1	1	24
C (31 or more treponemes)	39	6	0	33	0	5	1	33
Total	71	9	0	60	2	8	2	61

297220—54——2

tions in the IA test are reduced only from 6 to 5. However, since both the IA and TPI tests use the same virulent treponemes as antigen, and therefore may have the same base for presumptive specificity, the 6 serums giving A-zone reactions in the IA tests may be eliminated as probable syphilitics. If this is done, then the number of TPI positives is only reduced from 9 to 8. It is therefore apparent that differences may exist between the results of two or more tests using treponemal antigens in the same manner as happens between tests using beef-heart extractive antigen. It is also probable that maximum (A-zone) reactions with the IA test will be obtained in some instances when no other evidence of syphilis infection is present.

Conclusions

1. The pattern of reactivity of the IA test more closely follows the TPI test than that of the VDRL slide test.

2. Since the antibodies that produce positive reactions in the TPI and VDRL slide-type tests are known to be different, the substance producing such reactions in the IA test is probably similar to, if not identical with, the substance that activates the TPI test.

3. If the above conclusion is correct, then positive reactions in the IA test may be reflective of past rather than present syphilis infection.

4. Since the IA test is a simple, rapid laboratory procedure, and since antigen for this test need not be freshly prepared, this test may be a practical substitute technique for detecting the TPI test antibody.

Summary

1. The reactivity of the immune adherence (IA) test on 71 presumably nonsyphilitic patients and 234 patients with syphilis diagnoses is presented and discussed.

2. The relative reactivities of IA, TPI, and VDRL slide tests for syphilis in 305 previously diagnosed patients is shown and discussed.

REFERENCES

(1) Nelson, R. A., Jr.: *Treponema pallidum* immobilization test and the disappearance-adhesive phenomenon in syphilis. Presse méd. 60: 1719–1721 (1952).

(2) Nelson, R. A., Jr.: Changing concepts in the serodiagnoses of syphilis: Specific treponemal antibody versus Wassermann reagin. Brit. J. Venereal Diseases 28: 160–168 (1952).

(3) Nelson, R. A., Jr.: The immune-adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. Science 118: 733–737 (1953).

(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 (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 (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) Harris, A., Rosenberg, A. A., and Riedel, L. M.: A microflocculation test for syphilis using cardiolipin antigen. Preliminary report. J. Ven. Dis. Inform. 27: 169–174 (July 1946).

(8) Harris, A., Rosenberg, A. A., and Del Vecchio, E.: The VDRL slide flocculation test for syphilis. II. A supplementary report. J. Ven. Dis. Inform. 29: 72–75 (March 1948).