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Evaluation of the Qualitative and Automated Quantitative Microhemagglutination Assay for Antibodies to *Treponema pallidum*

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Qualitative and quantitative microhemagglutination assays for antibodies to *Treponema pallidum* (MHA-TP) were performed on 314 syphilitic and 597 presumably nonsyphilitic sera, and the results were compared with those of the fluorescent treponemal antibody-absorbed (FTA-ABS), the *Treponema pallidum* immobilization (TPI), and the Veneral Disease Research Laboratory (VDRL) tests. MHA-TP sensitivity was similar to that of the other tests in all stages of syphilis except primary syphilis, in which MHA-TP reactivity was only 64% compared with 82% in the FTA-ABS test, 73% in the VDRL test, and 67% in the TPI test. MHA-TP specificity was satisfactory and comparable to that of the other treponemal tests. Quantitation of the MHA-TP test was automated by use of Autotiter II equipment. Titers tended to become elevated later in the course of syphilis and to remain elevated longer than did VDRL titers. Reproducibility of the quantitative MHA-TP test was satisfactory, with duplicate tests agreeing within one doubling dilution on 97.5% of 351 reactive sera. Poor reproducibility was obtained with sera giving minimal reactions in the qualitative test, and such sera should be routinely retested. The MHA-TP is less time-consuming and costly than the FTA-ABS test and could be used in conjunction with the VDRL or another reagin test for syphilis to eliminate a large number of the FTA-ABS tests now required.

A passive hemagglutination test for syphilis in which an antigen derived from the virulent Nichols strain of *Treponema pallidum* is used has been described by Rathlev (6) and by Tomizawa and associates (7, 8). This test was adapted to an automated microhemagglutination procedure by Cox et al. (1), and in evaluations to date the automated procedure has yielded results comparable to those obtained with the fluorescent treponemal antibody-absorption (FTA-ABS) tests for syphilis (2, 4). Because a reliable hemagglutination test would possess a considerable advantage in time and cost over the manually performed FTA-ABS test or the *T. pallidum* immobilization (TPI) test, an evaluation of the manual and automated microhemagglutination assay for *T. pallidum* antibodies (MHA-TP) was undertaken and is reported here.

In this study, sera from patients in clinically defined categories of syphilis were tested by the Venereal Disease Research Laboratory (VDRL) test, the FTA-ABS test, the TPI test, and the MHA-TP test to evaluate the sensitivity of the hemagglutination test in comparison with other treponemal and reagin tests. Sera from presumably nonsyphilitic individuals were tested as a measure of test specificity. All MHA-TP reactors were quantitated by an automated method to determine the applicability of this procedure in a diagnostic laboratory.

MATERIALS AND METHODS

Test procedure. The MHA-TP test was performed according to the provisional technique (11) except that all absorbed sera were held at 4 to 6 C for 16 to 24 hr before testing, to permit both qualitative and quantitative testing on the same batch of absorbed serum. All sera were tested by the qualitative procedure at a 1:80 final serum dilution; sera giving a reaction of one plus or greater were then tested quantitatively by use of serial twofold dilutions from 1:80 through 1:2,560. Quantitative tests were then repeated on a second day to measure reproducibility.

The FTA-ABS and VDRL tests were performed

according to the published techniques (9). The TPI test was performed as in the described procedure (10) with the following exceptions: undiluted guinea pig complement was used instead of 200 hemolytic units; results were reported as reactive with greater than 60% immobilization, weakly reactive with 30 to 59%, inconclusive with 18 to 29%, and nonreactive with less than 18% immobilization.

Reagents. Reagents for many of the MHA-TP tests were generously supplied by Canalco, Inc. Antigen for the FTA-ABS test was prepared in this laboratory (12). All other reagents were obtained from commercial sources.

Equipment. The quantitative MHA-TP tests were performed with the use of an Autotiter II (Canalco, Inc., Rockville, Md.) to prepare serial dilutions of serum and to add sensitized cells.

Sera. Syphilitic sera were obtained from 314 well-documented cases of syphilis, which were diagnosed on the basis of clinical and historical, as well as laboratory, data, and from 51 patients with dark-field positive lesions on whom additional clinical information was not available.

Biological false-positive (BFP) specimens were obtained from 172 patients without signs or symptoms of syphilis, whose sera were reactive in the VDRL test and nonreactive in the FTA-ABS and TPI tests, and from 22 narcotic users with reactive VDRL tests and nonreactive FTA-ABS tests.

Four hundred twenty-five presumed normal sera, obtained from a local blood bank, were selected on the basis of a nonreactive VDRL test; 96 additional unclassified specimens were selected from sera submitted to this laboratory for tests for syphilis.

Sera were coded, randomized, and stored at −20 C.

RESULTS

MHA-TP and other treponemal and reagin tests were performed on 314 syphilitic sera. Two sera (0.6%) gave nonspecific agglutination of the unsensitized control cells and were excluded from further testing. The results obtained on the 312 sera with valid hemagglutination reactions are given in Table 1. The MHA-TP test was comparable in reactivity to the other treponemal tests in all categories of syphilis except primary syphilis, in which it was considerably less sensitive than the FTA-ABS test. To test further the apparent lack of sensitivity of the MHA-TP test in primary syphilis, sera were obtained from 51 additional patients with dark-field positive lesions of primary syphilis. The MHA-TP test was reactive in only 27 (53%) of these patients compared with 36 (71%) VDRL reactors and 40 (78%) FTA-ABS reactors.

TABLE 1. *Reactivity of serological tests on 312 syphilitic sera*

Stage of syphilis	No. of sera tested	Per cent reactive			
		VDRL	FTA-ABS	TPI	MHA-TP
Primary	63	73	82	67	64
Secondary	43	100	100	100	96
Early latent ...	53	100	98	96	96
Late latent ...	87	93	98	97	97
Late Symptomatic	66	94	100	98	98

MHA-TP and FTA-ABS test results on the syphilitic sera presented in Table 1 agreed as follows: 70% in primary syphilis, 95% in secondary syphilis, and 98% in all other categories of syphilis. In all cases in which the two tests disagreed, the MHA-TP was less sensitive than the FTA-ABS test. MHA-TP and TPI test results agreed as follows: 87% in primary syphilis, 95% in secondary syphilis, 97% in latent syphilis, and 100% in late symptomatic syphilis. When the two tests disagreed, neither test was consistently more reactive than the other, although there was a tendency in early syphilis for the TPI test to be more sensitive than the MHA-TP test; 13 sera from patients with primary or secondary syphilis were reactive only in the TPI test compared with 5 such sera reactive only in the MHA-TP test.

MHA-TP and FTA-ABS tests were compared on 96 sera submitted to this laboratory for FTA-ABS testing. Clinical data are not available on these patients. The two tests agreed on 89 sera and disagreed on 3 sera (1 was reactive only in the MHA-TP test, and two, only in the FTA-ABS test). On four sera that were nonreactive in the FTA-ABS test, valid MHA-TP results could not be obtained because of nonspecific agglutination of control cells.

BFP specimens were obtained from 172 patients without clinical or historical evidence of syphilis whose sera were reactive in the VDRL test and nonreactive in the FTA-ABS and TPI tests. Five sera (3%) were eliminated because of nonspecific agglutination of control cells. The MHA-TP test was reactive in 2 (1.2%) of the 167 sera yielding valid test results (Table 2). The BFP group included sera from nine narcotic users; all were nonreactive in the MHA-TP test. An additional 22 sera from VDRL reactive, FTA-ABS nonreactive narcotic users were tested, and 21 were nonreactive in the MHA-TP test; valid MHA-TP results could not be obtained with 1 serum because of nonspecific agglutination of control

TABLE 2. *Reactivity of serological tests on presumably nonsyphilitic sera*

Category	No. of sera tested	Per cent reactive			
		VDRL	FTA-ABS	TPI	MHA-TP
BFP	167	100	0	0	1.2
Narcotic users	21	100	0	NT[a]	0
Normal	424	0	NT	NT	0.9

[a] Not tested.

cells.

The "normal" group consisted of 425 VDRL nonreactive sera obtained from a local blood bank. These sera were tested by the MHA-TP test, and all reactors were further tested by the FTA-ABS test. One serum (0.2%) was eliminated because of nonspecific agglutination of control cells. Of 424 "normal" sera with valid hemagglutination results, four (0.9%) were reactive in the MHA-TP test (Table 2); all four MHA-TP reactors were also FTA-ABS reactive.

Quantitation of the MHA-TP test was automated by using Autotiter II equipment to prepare serial dilutions of serum and to add cells. Quantitative MHA-TP and VDRL tests were done on all reactive sera. Titer patterns obtained in the various clinical categories of syphilis are presented in Fig. 1. MHA-TP titers tended to be elevated later in the course of the disease and to remain higher longer than did VDRL titers. In the VDRL test, nearly 75% of the sera from patients with secondary syphilis had high titers ($\geq$1:32), but in the early latent group titers tended to be evenly distributed across the range of dilutions tested, and a definite shift toward lower VDRL titers was observed in the late latent and late symptomatic groups.

In the MHA-TP test, on the other hand, the greatest proportion of high titers ($\geq$1:2,560) was observed in the early latent group. MHA-TP titers tended to be more evenly distributed in the late latent and late symptomatic groups, but never showed the trend toward lower titers seen in the VDRL test.

Reproducibility of the MHA-TP test was measured by comparing: (i) the qualitative test result and the result on the comparable dilution in the quantitative test, performed on the same and different days, and (ii) quantitative test results obtained on two different days.

The ability of the quantitative test to reproduce the qualitative test result was positively correlated with the degree of reactivity in the qualitative test (Table 3). The quantitative test performed on the same day as the qualitative test was reactive with 99% of the sera which had given a three or four plus reaction in the qualitative test and with 94% of the two plus reactors, but with only 44% of the one plus reactors. When the quantitative test was performed on a different day from the qualitative test, it was reactive with 99% of the three and four plus reactors, 96% of the two plus reactors, and 36% of the one plus reactors.

Duplicate test results were obtained on 351 reactive sera tested by the automated quantitative MHA-TP test on two different days. The two titers were identical on 241 sera (68.7%), and agreed within one doubling dilution of each other on an additional 101 sera (28.8%). Thus, the two quantitative test results agreed within one dilution of each other on 97.5% of the sera tested.

DISCUSSION

The MHA-TP test offers advantages over the FTA-ABS and TPI tests in the amount of staff time required for manual testing and in the cost of necessary equipment and facilities. The test can be performed with commercial reagents, qualitatively or quantitatively, and with standard Microtiter equipment; certain steps in the quantitative procedure can be automated with commercially available instruments.

In the evaluation reported here, the MHA-TP test compared favorably with the TPI and FTA-ABS tests in all stages of syphilis except primary syphilis, in which it was less sensitive than the FTA-ABS test and possibly less sensitive than the TPI test. Other investigators have also noted a lack of sensitivity in the hemagglutination test in primary syphilis (2, 3, 8). In a recent field study of the MHA-TP test, the three participating laboratories reported MHA-TP reactivities in primary syphilis of 92.5% on 134 sera, 75% on 32 sera, and 94.7% on 19 sera (2). These MHA-TP results show a higher percentage of reactivity than the 64% found in our laboratory, but the FTA-ABS test was also more reactive in the other laboratories, suggesting a difference in the infection status of the patient groups categorized as primary syphilis. More information is needed on the dynamics of antibody detection by the various treponemal tests in first-infection pri-

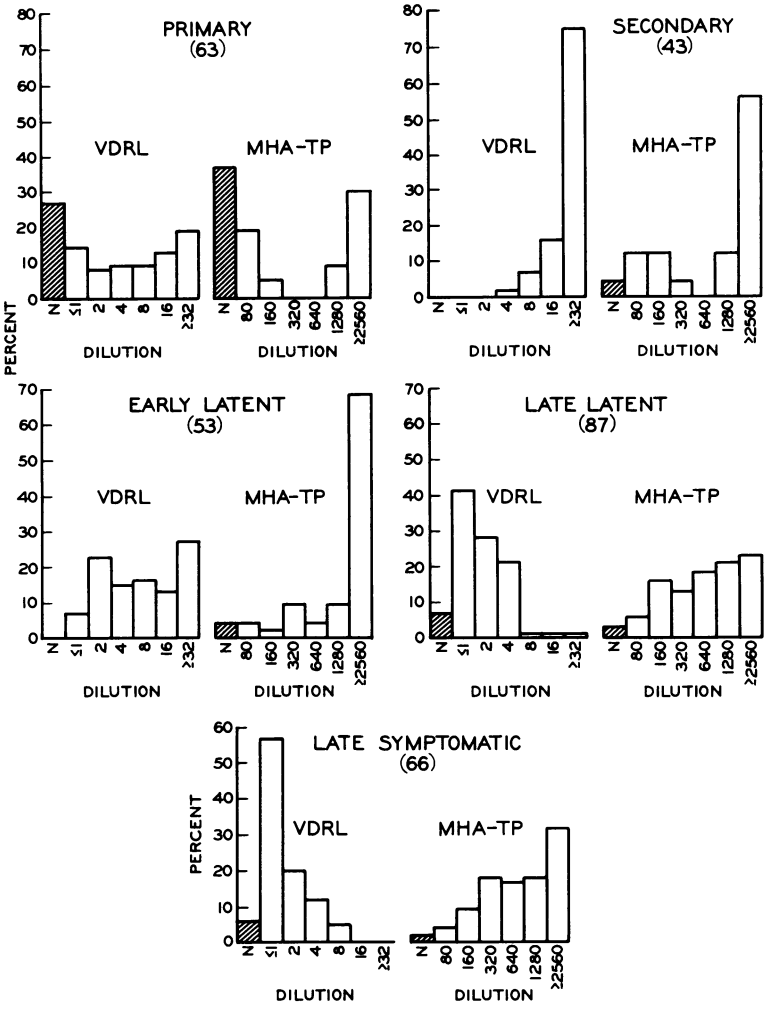

FIG. 1. *Percentage distribution of titers in the VDRL and MHA-TP tests in the various categories of syphilis. Numbers in parentheses are the number of patients in each category. Dilutions are expressed as the reciprocal of the highest serum dilution giving a positive result; N is negative.*

TABLE 3. *Agreement of qualitative and quantitative MHA-TP test results on reactive sera tested on the same day and on different days*

Qualitative test result (in pluses)	Quantitative tests					
	Same day			Different day		
	Total sera	Reactive		Total sera	Reactive	
		No.	Per cent		No.	Per cent
1	46	20	44	47	17	36
2	90	85	94	92	88	96
3 or 4	265	262	99	270	268	99
Total	401	367	92	409	373	91

mary syphilis. In five rabbits experimentally infected with *T. pallidum*, Tomazawa et al. (8) found that immunofluorescent activity invariably preceded hemagglutinating activity, whereas Cox and her associates (1) reported on nine rabbits in which hemagglutins were detected as early or earlier than immunofluorescent activity. Okamoto and Tanabe (5) examined rabbit immunoglobulins reacting in the hemagglutination test and found that these were exclusively in the 19*S* fraction of sera taken 11 days after experimental infection. Data of this kind, including a characterization of the antigens and quantitation of the immunoglobulins involved, are needed for human

syphilis.

The value of quantitative MHA-TP results is not resolved. Certain trends are evident in the various categories of syphilis, but information on the reactivity of sera from individual patients over a period of time is needed to evaluate this procedure fully. The few patients' sera that we examined in this way suggest that MHA-TP titer movement parallels that of the FTA-ABS test, in that the titer response to treatment is slower and less pronounced than in the VDRL test.

The specificity of the MHA-TP test was good, with a specificity of 98.8% on BFP sera. In the presumed normal group, the reactivity of the MHA-TP was only 0.9%, and all MHA-TP reactive sera were also reactive in the FTA-ABS test.

The reproducibility of the automated quantitative MHA-TP test was satisfactory. The poorest reproducibility was obtained when sera reacting minimally in the qualitative test were retested quantitatively. Many of these sera yielded weak nonspecific agglutination of control cells upon retesting rather than negative reactions. These results suggest that good laboratory practice would encompass the retesting of all one and two plus qualitative reactors before results are reported.

Because of the apparent lack of sensitivity of the MHA-TP test in primary syphilis, we would hesitate to use this test as the only treponemal procedure at this stage of its development. However, it could provide a very useful tool in conjunction with the VDRL test or another reagin test to eliminate a large number of the FTA-ABS tests now required. A reaction in both the reagin and MHA-TP tests would indicate syphilis, and no further testing would be required. Reagin reactive, MHA-TP nonreactive sera would require FTA-ABS testing.

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