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Specificity, Sensitivity, and Reproducibility Among the Fluorescent Treponemal Antibody-Absorption Test, the Microhemagglutination Assay for *Treponema pallidum* Antibodies, and the Hemagglutination Treponemal Test for Syphilis

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Using 920 sera, we compared the specificity and reproducibility of the hemagglutination treponemal test for syphilis with those of the fluorescent treponemal antibody-absorption test and the microhemagglutination assay for *Treponema pallidum* antibodies; we found all three tests to be comparable. However, the hemagglutination treponemal test for syphilis, like the microhemagglutination assay for *T. pallidum* antibodies, lacked sensitivity in sera from patients with primary syphilis.

In a previous study (3), our laboratory evaluated two commercially available treponemal antibody kits for diagnosing syphilis. One kit, which used turkey erythrocytes as the carrier for the *Treponema pallidum* antigen (6), was found to be less sensitive in sera from patients with all stages of syphilis than the fluorescent treponemal antibody-absorption (FTA-ABS) test. The other hemagglutination test, the microhemagglutination assay for *T. pallidum* antibodies (MHA-TP) (1, 5), although slightly less sensitive in sera from patients with early primary syphilis (2, 3), was as sensitive as the FTA-ABS test in sera from patients with other stages of syphilis and was as specific as the FTA-ABS test in sera from nonsyphilitics.

In 1978, the hemagglutination treponemal test for syphilis (HATTS) was described (9). *T. pallidum* antigen, coupled with turkey erythrocytes, is also used as the antigen in this hemagglutination test. The initial evaluation (9) and one additional evaluation (4) indicated that the HATTS was comparable to the MHA-TP.

In the present study, the sensitivity, specificity, and reproducibility of the HATTS were compared with those of the MHA-TP, the FTA-ABS test, and the Venereal Disease Research Laboratory (VDRL) slide test, a nontreponemal test.

MATERIALS AND METHODS

Sera. For the evaluation of the HATTS, two groups of sera were used. One group consisted of 439 frozen sera from the Venereal Disease Serology Laboratory Serum Bank, Centers for Disease Control, Atlanta, Ga., and the other group consisted of 481 fresh sera from the DeKalb County, Ga., Sexually Transmitted Diseases Clinic. Most of the syphilitic sera from the sexually transmitted diseases clinic were from patients who were treated either the week before or on the day their sera were drawn. Therefore, no attempt was made to separate these sera into treated or untreated categories.

To assess the reproducibility of the HATTS, we dispensed each of six sera into 10 portions and coded them so that they would be included with the routine testing of the frozen sera over 5 consecutive testing days and with five periodic testings throughout the study. These six sera were selected so that two were reactive at a 3 to 4+ level in the FTA-ABS test, but reactive only at a 1 to 2+ level in the MHA-TP (both from patients with primary syphilis), two were highly reactive in both the MHA-TP and the FTA-ABS test (both from patients with latent syphilis), and two were reactive only in the VDRL test (both from patients with false-positive serological results).

Serological testing. Frozen sera and sera for reproducibility testing were coded so that the technicians who performed the tests did not know the category to which these sera had been assigned. Fresh sera were tested before clinical histories were taken. The VDRL and FTA-ABS tests were done with Centers for Disease Control reference reagents and by standard techniques (8). The MHA-TP was performed with Sera-Tek kits (Fujizoki Pharmaceutical Co., Ltd., Tokyo, Japan; distribution by Ames Company [Division of Miles Laboratories, Inc., Elkhart, Ind.]) in accordance with the directions of the manufacturer. The HATTS was performed with kits supplied by the manufacturer (Difco Laboratories, Detroit, Mich.) and

in accordance with the proposed product profile. Briefly, sera to be tested were heated for 30 min at 56°C and then diluted 1:16 in test diluent. Next, 25 μl of each of these sera was placed in microtitration tray wells in duplicate. Sensitized erythrocytes were added to one well, and unsensitized erythrocytes were placed in the second well. The final dilution of serum plus cells was 1:80. Plates were incubated undisturbed for 1 h at 26 ± 3°C. Readings of the HATTS results were based on the intensity of the hemagglutination pattern. Sera with readings of 1+ to 4+ were recorded as reactive. Those sera read as ± were retested. Sera which were twice read as ± and negative sera were recorded as nonreactive. Sera reacting with the unsensitized erythrocytes were considered unsatisfactory. Before being used in the study, the hemagglutination reagents were tested with reference sera both in our laboratory and in that of the Centers for Disease Control Biological Products Division. Except where specified, a single lot of each hemagglutination kit was used throughout the study. Four serologists were involved, and each was responsible for the performance of a single test. The results were compiled at the end of each day of testing. Control sera were assayed daily by all tests.

RESULTS

A total of 920 sera were tested in the study. Tables 1 and 2 show the test results for the 592 nonsyphilitic sera. Twelve of these sera were known to be from patients with diseases other than syphilis, including leprosy, lupus, and malaria. Of these 12 sera, 2 were reactive in the FTA-ABS test only and 1 was weakly reactive in the VDRL test. The possible causes of the other false-positive reactions are unknown. Of the 22 sera which were false-positive in the FTA-ABS test, 13 were read as 1+, 6 as 2+, and 3 as 3+. Of the 11 sera which were false-positive in the HATTS, 7 reacted at a 1+ level, 2 at 2+, and 2 at 3+. Both of the sera which were false-positive in the MHA-TP were reactive at a 1+ level. For all sera, fresh or frozen, which gave unequivocal results, there were no statistically significant differences in specificities among the results of the HATTS, MHA-TP, FTA-ABS, and VDRL tests. However, if sera which reacted in more than one test were excluded from the calculations, then there were significant differences, in fresh sera only, between the results of the FTA-ABS test and those of the HATTS ($P < 0.001$) or the VDRL test ($P < 0.01$). There was a significant difference between the results of the MHA-TP and those of the VDRL test ($P < 0.03$), but there were no significant differences between HATTS results and those of the MHA-TP or the VDRL test. The significant difference between the FTA-ABS test results for frozen sera and the FTA-ABS test results for fresh sera ($P < 0.02$) is of interest. Among the other three tests, no significant differences in specificity existed between the results for fresh sera and those for frozen sera.

TABLE 1. *Test results for sera from patients without syphilis*

Test result	No. (%) of frozen sera	No. (%) of fresh sera
Seronegative	182 (91.0)	354 (90.3)
False-positive		
HATTS positive	3 (1.5)	3 (.8)
MHA-TP positive		
FTA-ABS positive	1 (.5)	17 (4.3)
VDRL positive	1 (.5)	5 (1.3)
HATTS and FTA-ABS positive	2 (1.0)	1 (.25)
HATTS and MHA-TP positive	1 (.5)	1 (.25)
VDRL and FTA-ABS positive	1 (.5)	
Equivocal test result		
VDRL weakly reactive	3 (1.5)	7 (1.8)
FTA-ABS borderline	6 (3.0)	4 (1.0)

TABLE 2. *Specificity based on reactivity*

Test	Specificity (%) based on reactivity in:			
	One or more tests		Only one test	
	Frozen sera[a]	Fresh sera[b]	Frozen sera[c]	Fresh sera[d]
HATTS	96.9	98.7	98.4	99.2
MHA-TP	99.5	99.7	100	100
FTA-ABS	97.9	95.3	99.5	95.5
VDRL	99.0	98.7	99.5	98.7

[a] Based on 191 sera.
[b] Based on 381 sera.
[c] Based on 187 sera.
[d] Based on 379 sera.

The results of tests on the 328 syphilitic sera are shown in Table 3. Of the primary syphilitic sera which were nonreactive in the treponemal tests, at least 50% were from dark-field-positive chancres. Of the 79 primary syphilitic sera, 2 were nonreactive in the FTA-ABS test only, 2 were nonreactive in the HATTS only, 8 were nonreactive in both the HATTS and the MHA-TP, and 1 was nonreactive in the MHA-TP only. All sera from patients with secondary syphilis were reactive in all treponemal tests. Those nonreactive in the VDRL test were from patients with treated secondary syphilis. In the latent category, one serum was nonreactive in the MHA-TP only, and one serum was nonreactive in the FTA-ABS test only. In addition to these nonreactive sera, two sera from patients categorized as having late cardiovascular syphilis were nonreactive in both hemagglutination tests. Since treatment affects the reactivity of the VDRL test, this test was not included in the calculations for sensitivity. The only significant differences in sensitivity existed between the

TABLE 3. *Test results for sera from patients with syphilis*

Sera from patients at indicated stage	HATTS		MHA-TP		FTA-ABS		VDRL		
	Reactive	Nonreactive	Reactive	Nonreactive	Reactive	Nonreactive	Reactive	Weakly reactive	Nonreactive
Primary[a]									
Untreated frozen	26	4	26	4	30		26		4
Treated frozen	24	2	24	2	24	2	22		4
Fresh	19	4	20	3	23		14	1	8
Secondary[b]									
Untreated frozen	33		33		33		33		
Treated frozen	42		42		42		36	2	4
Fresh	14		14		14		10	1	3
Latent[c]									
Untreated frozen	34		34		33	1	31	3	
Treated frozen	43		42	1	43		39	1	3
Fresh	27		27		27		17	8	2
Cardiovascular[d]	19	2	19	2	21		10	3	8
Neurosyphilitic[e]	10		10		10		9		1
Past syphilis[f]	25		25		25		6	6	13

[a] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with primary syphilis were 87.3, 88.6, 97.5, and 79.7%, respectively.

[b] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with secondary syphilis were 100, 100, 100, and 88.8%, respectively.

[c] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with latent syphilis were 100, 99, 99, and 95.2%, respectively.

[d] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with cardiovascular syphilis were 89.5, 89.5, 100, and 38.1%, respectively.

[e] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with neurosyphilis were 100, 100, 100, and 90%, respectively.

[f] Sensitivities of the HATTS, MHA-TP, FTA-ABS, and VDRL tests in sera from patients with past syphilis were 100, 100, 100, and 48%, respectively.

results of the FTA-ABS test and those of the MHA-TP ($P < 0.04$) and HATTS ($P < 0.02$) with sera from patients with the primary stage of syphilis. There was no significant difference between sensitivities of the HATTS and the MHA-TP in sera from patients with this stage of syphilis. Of the 920 sera tested, there were only 2 reactions with nonsensitized control cells in the HATTS and 5 in the MHA-TP. Such reactions, of course, invalidate the test unless the sera can be diluted or absorbed to eliminate the reactions.

Each of six sera were tested 10 times; reproducibility, as shown in Table 4, was approximately the same for the HATTS, the FTA-ABS test, and the VDRL test. Only the MHA-TP was 100% repeatable. A gray area of reading occurred in all three tests, which we designated as N-± and ±-1 for the two hemagglutination tests. These sera were retested before a final reading was recorded. Table 5 shows the results of the retesting of these sera and sera which were initially either nonreactive or reactive at a 1+ level. In the MHA-TP, all questionable sera were read on repeat testing as nonreactive, whereas 88% of the 22 sera falling in the gray area on the first reading were reported as nonreactive upon repeat testing in the HATTS. These nonreactive results agreed with the clinical diagnoses.

On each of the 27 testing days, reactive control sera were quantitated for both the MHA-TP and the HATTS. For the MHA-TP, the endpoint titer was 1:5,120 for 19 days, 1:2,560 for 4 days, and 1:10,240 for 4 days. For the HATTS with the control sera used for the first 21 days of the study, the endpoint titer was 1:5,120 for 14 days and 1:2,560 for 7 days. For the last 6 days, a new control serum was used for the HATTS. The endpoint titer was 1:1,280 for 3 days and 1:640 for 3 days. To assure ourselves that the HATTS did not vary depending on the lot of reagents, we used a second lot of HATTS kit reagents to retest 75 randomly selected sera. Of the 75 sera, only 2 changed in reactivity, 1 be-

TABLE 4. *Reproducibility studies*

Sera from patients at indicated stage	No. of sera showing the indicated reactivity																						
	HATTS					MHA-TP					FTA-ABS						VDRL						
	4+	3+	2+	1+	N[a]	4+	3+	2+	1+	N	4+	3+	2+	1+	B[b]	N	R_{32}[c]	R_{16}	R_8	R_2	R_1	W_0[d]	N	Contaminated
Primary																							
Untreated			1	2	7			7	3		4	3	3							3	5			2
Treated			8	2				4	6		2	3	5				6	4						
Latent																							
Untreated	9			1		6	1	2	1		7		3							2	1			7
Treated	10					10					9		1							1	4	2		3
False-positive sera																							
Group 1					10					10					9	1	4	3	3					
Group 2					10					10					1	9				3	4	3		

[a] N, Nonreactive.
[b] B, Borderline.
[c] R, Reactive; subscript numeral indicates titer.
[d] W, Weakly reactive; subscript numeral indicates titer.

TABLE 5. *Repeat readings of fresh sera giving questionable reactions*

Repeat reading	First reading (no. of sera)			
	N-±[a]	N[b]	±-1[a]	1+
HATTS				
N	14	43	5	
1+	2		1	6
MHA-TP				
N	4	15	1	
1+				5

[a] See text for explanation.
[b] N, Nonreactive.

coming nonreactive and 1 becoming reactive; there was 97.3% agreement between lots of reagents.

DISCUSSION

From this comparative testing, it appears that the sensitivity of the HATTS is similar to that of the MHA-TP, and both are less sensitive than the FTA-ABS test in sera from patients with primary syphilis (3, 7). This study also reemphasizes our earlier findings that the MHA-TP and the FTA-ABS test (3) and now the HATTS are not any more specific than the nontreponemal tests when used to screen populations. The higher rate of nonspecificity of the FTA-ABS test for fresh sera cannot be explained at this time. However, all 17 sera which reacted in this manner were retested in another laboratory, and the original test results were confirmed. Presently, we cannot recommend any of these three treponemal tests as screening tests.

Reproducibility of the HATTS is comparable to those of the FTA-ABS and VDRL tests. The HATTS appears to be less reproducible than the MHA-TP. However, this may reflect our choice of an untreated primary serum for testing reproducibility or our unfamiliarity with the test rather than the reproducibility of the test itself. We suggest that all sera falling in the N-±—±-1 range be retested before the final report is made.

The HATTS kit is no more complicated to use than the MHA-TP kit and has the advantage of a 1-h incubation period, as opposed to the 4-h incubation period required for the MHA-TP. In our study, we did not attempt to evaluate the stability of the reconstituted hemagglutination reagents, but we suggest that the reconstituted cells in both the MHA-TP and the HATTS be used within 3 days.

During the testing period, five HATTS kits showed unexpected autoagglutination, three times with the sensitized cells and twice with the unsensitized cells. We could not determine the source of this autoagglutination, but apparently, we eliminated the problem empirically by using all disposable laboratory ware for preparing and dispensing the cells and sera.

On the basis of our data and the experience of Wentworth et al. (9) and Peter et al. (4), we concluded that the HATTS appears to be as valuable as the MHA-TP as a test for confirming syphilis. However, the FTA-ABS test is still necessary for those sera with unsensitized control-cell reactions and may be preferred for confirming primary syphilis and late cardiovascular syphilis.

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