Evaluation of the Automated Micro-Hemagglutination Assay for Antibodies to *Treponema pallidum*

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THE quantitative automated micro-hemagglutination assay for antibodies to *Treponema pallidum* (AMHA-TP) described by Cox and co-workers (*1*) appears to offer several technical advantages over the treponemal tests currently in use. The AMHA-TP assay is easily done, requires small amounts of reagents, and makes practicable the quantitation of treponemal antibody. In a preliminary evaluation at the National Communicable Disease Center's Venereal Disease Research Laboratory (*2*), satisfactory agreement was seen with the fluorescent treponemal antibody absorption (FTA-ABS) test and with clinically determined diagnostic categories.

This study was undertaken to determine the value of the AMHA-TP assay as an aid to diagnosis, to determine the reproducibility of its titer, and to evaluate its use in a public health laboratory. The AMHA-TP assay results on 900 serums were compared with the FTA-ABS test results and correlated with clinical diagnoses.

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Materials and Methods

The automated quantitative micro-hemagglutination assay for *T. pallidum* antibodies, the fluorescent treponemal antibody absorption, and Venereal Disease Research Laboratory (VDRL)

slide tests were all done according to published techniques (*3,4*). The automation of the AMHA-TP assay entailed use of an Autotiter system (*A*) and reagent kit (*B*).

In this study 900 specimens were examined. Most of these specimens were selected from samples submitted by mail for routine examination. Selection was based on reactivity to the VDRL slide and FTA-ABS tests, clinical history, and adequacy of volume.

The clinical histories were obtained by one of two methods. Either a brief form outlining the information desired was sent to the physician submitting the specimen or the information was taken from the files of the venereal disease investigation unit.

The remaining specimens, from patients with diseases or conditions known to produce false positive reactions to tests for syphilis, were drawn by request. Test serums were classified into two clinically defined categories: syphilitic and nonsyphilitic.

The 600 nonsyphilitic specimens included 300 serums from premarital or pregnant donors. Although 200 of the serums were from persons with no history of syphilis and were not reactive to FTA-ABS tests, they reacted positively to the VDRL slide test. The remaining 100 serums were from patients with diseases known to cause false positive reactions to tests for syphilis or persons whose serums reacted positively to a VDRL slide test although there was no probable clinical cause. The 300 syphilitic specimens included serums from persons in each of the four stages of syphilis.

Reproducibility studies were done on 40 serums tested in duplicate on two different days. Of these, 30 were from syphilitic and 10 were from nonsyphilitic donors. An additional 10 specimens from syphilitic donors were tested in the same manner before and after storage at —20°C. for a minimum of 5 days.

Table 1. Reactivity of the automated micro-hemagglutination assay (AMHA-TP) and the fluorescent treponemal antibody absorption (FTA-ABS) test on 900 specimens

Clinical category	Number of specimens	Reactive to AMHA-TP assay		Reactive to FTA-ABS test	
		Number	Percent	Number	Percent
Nonsyphilitic: presumed normal	300	0	0	0	0
VDRL reactive and FTA-ABS negative	200	0	0	0	0
False positive	100	7	7.0	8	8.0
Syphilitic:					
Congenital	17	16	94.1	16	94.1
Primary	19	18	94.7	19	100.0
Secondary	23	23	100.0	23	100.0
Tertiary	10	9	90.0	10	100.0
Latent	194	184	94.8	186	95.9
Not specified	37	36	97.3	35	94.6
Total	900	293	32.6	297	33.0

Results and Discussion

Agreement between the AMHA-TP assay and FTA-ABS test was 99.8 percent for nonsyphilitic specimens, 98.3 percent for syphilitic specimens, and 99.3 percent overall. Table 1 shows that agreement between tests was close in each clinical category with slightly higher reactivity seen with the FTA-ABS test.

Disagreement of test results occurred with six specimens. Five were reactive in the FTA-ABS and nonreactive in the AMHA-TP. Of the specimens that reacted to the FTA-ABS test, one was from a 22-year-old woman with no history or signs of syphilis, one was from a patient with primary syphilis, one was from a 77-year-old man with tertiary syphilis who had an Argyll-Robertson pupil reaction during a routine eye examination, and two were from patients with latent syphilis. The latter two patients with syphilis were a 27-year-old man and a 64-year-old man whose disease was diagnosed as in the latent stage 9 years previously. The only specimen reactive in the AMHA-TP assay and nonreactive in the FTA-ABS test was from a patient who had reported syphilis 23 years previously.

The false positive category included eight specimens reactive to the FTA-ABS test. Of these, seven were also reactive in the AMHA-TP assay. The causes of the reactions were attributed to diseases other than syphilis in five instances and were unknown in three. All eight specimens were reactive to the VDRL slide test. Diagnoses of the patients' illnesses, their ages, and test results are shown in table 2. If reactivity to the FTA-ABS test is considered evidence of syphilis, then all specimens listed in table 2 could be excluded from the false positive category. Unfortunately, no information other than that on the form sent in by

their physicians was available concerning these eight patients.

The range of titers observed with the AMHA-TP assay and the mean titer for each clinical category of syphilis can be seen in table 3. No relationship could be determined between the height of the titer and stage of disease because of the range and overlap of titers. These results confirm the findings of Logan and Cox (*2*).

Of the 40 specimens tested to determine the reproducibility of the AMHA-TP assay, 22 were in absolute agreement, 15 show a range of one doubling dilution, and three a range of two doubling dilutions. The reproducibility of titers was 92.5 percent which is below the accepted level of 95 percent (*5*). All nonsyphilitic specimens showed absolute agreement. No consistent change in AMHA-TP assay titer was observed when 10 serums were assayed before and after storage at —20°C. for a minimum of 5 days.

Repeat testing of serums showing a ± reaction at the 1:80 dilution was intended to confirm the test result. Of the eight serums retested, four of the reactions remained at the same level, two definitely reacted at the 1:80 titer, and the remaining two showed a titer of 1:160. Of the four blood samples showing a titer, one was from a patient with latent syphilis and the remaining three were from patients with primary syphilis. The four specimens showing no change in titer were from nonsyphilitic donors. Acceptance of the initial test result would have decreased the agreement between tests in the primary syphilis category from 94.7 percent to 78.9 percent. The cause of these differences in test results could not be determined.

The AMHA-TP assay provisional technique (October 1, 1969) required the use of preabsorbed reactive and nonreactive controls and absorbing diluent checked at the time of production only for standard ability to remove nonspecific antibodies (*3*). The addition of a nonspecific control and assaying of all controls before and after absorption at the time of testing would have served as a more effective control system.

We understand that reagents for both the reactive and nonreactive controls are now supplied unabsorbed, and the preabsorbed reagents are no longer available. The 1969 provisional technique has been superseded by "Automated, Quantitative Micro-Hemagglutination Assay for *Treponema Pallidum* Antibodies (1,2,3,6) (AMHA-TP)," February 16, 1971.

Although 0.075 ml. of the diluted (1:6.5) antigen is required per test, the volume required to fill the manifold system varies from 35 to 40 ml. Some of this material, approximately 10 ml., can be recovered from the manifold each day for use on the following day only. However, at the end of the week the total volume must be discarded. On a weekly basis a minimum of 135 to 160 ml. of reagent in excess of testing material would be required. The cost per test would depend on the number of specimens tested per run.

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Table 2. Test results of 8 specimens reactive to the fluorescent treponemal antibody absorption test

Clinical diagnoses	Age of patient (years)	Automated micro-hemagglutination assay titer	Fluorescent treponemal antibody absorption test [1]	Venereal Disease Research Laboratory slide test [2]
Liver disease	66	160	1+	4
Pregnancy and diabetes	37	320	3+	1
Scleroderma	26	5,120	4+	4
Diabetes	86	80	1+	1
Malaria	93	320	4+	32
Unknown	55	1,280	2+	([3])
Unknown	61	640	2+	1
Unknown	22	0	1+	([3])

[1] Numbers represent degrees of fluorescence.
[2] Reported in dils.
[3] Weakly reactive.

Table 3. Automated micro-hemagglutination assay titers on serum from patients with syphilis

Clinical category	Number of specimens	Range of titer	Mean titer
Congenital	17	0–5,120	1,445
Primary	19	0–5,120	914
Secondary	23	80–20,480	2,348
Tertiary	10	0–5,120	1,504
Latent	194	0–40,960	1,639
Not specified	37	0–40,960	2,679
Total	300	.....	.....

electronic problems were encountered in performing the serial dilutions with the Autotiter. Technical errors made by the machine were not always easily detected and close monitoring was necessary. Further adjustments in the manufacture of the Autotiter since this study was completed are said to have eliminated most or all of these problems.

Conclusion

No advantage was apparent in performing a quantitative rather than a qualitative treponemal test as an aid to diagnosis. Further study of the titer response to patient treatment may prove quantitation a useful tool in a public health laboratory.

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(*3*) U.S. Public Health Service: Automated, quantitative micro-hemagglutination assay for *Treponema pallidum* antibodies (1, 2, 3) (AMHA–TP) (Provisional technique). National Communicable Disease Center, Atlanta, Ga., Oct. 1, 1969. Processed.

(*4*) U.S. Public Health Service: Manual of tests for syphilis, 1969. PHS Publication No. 411. U.S. Government Printing Office, Washington, D.C., 1969.

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EQUIPMENT REFERENCES

(*A*) Canalco, Inc., 5635 Fishers Lane, Rockville, Md. 20852.

(*B*) Fuji-Zoki Pharmaceutical Co., Ltd., 724, 2-Chome, Shimo-Shinjyuku-Ku, Tokyo, Japan.

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The trend in syphilis serology has been the increased use of treponemal tests. Because the tests currently in use are complex and time consuming, the simplicity of the quantitative automated micro-hemagglutination assay for antibodies to *Treponema pallidum* (AMHA-TP) makes it appear well suited for use in a public health laboratory.

Nine hundred serums from clinically defined donors were tested with the fluorescent treponemal antibody test and the quantitative automated micro-hemagglutination assay. Close agreement was seen between the results of the two types of tests.

No relationship was seen between the AMHA-TP assay titers and the clinical category of the disease. Reproducibility of serum titers was estimated at 92.5 percent. Freezing did not appear to affect the antibody titer.

Changes were suggested in the test procedure concerning the use of controls and testing of serums showing minimal results at the initial dilution.

Although no advantage was seen in using a quantitative treponemal test, further study of titer response to patient treatment may prove this procedure to be a useful tool.