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Fluorescent Treponemal Antibody Absorption Double-Staining Test Evaluation

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The fluorescent treponemal antibody absorption (FTA-ABS) double-staining (DS) test has been developed for microscopes equipped with incident illumination, and the procedure offers many advantages over the FTA-ABS test when tests are performed with this equipment. In this study, 346 fresh sera, including 35 from patients with syphilis, were evaluated by the FTA-ABS DS test. Parameters for investigation included two readers, each using a different microscope; a new FTA-ABS DS test reporting system; sera heated at 56°C for 30 min versus unheated sera; and sera retested after at least 2 weeks of freezer storage. Agreement for FTA-ABS DS test readings between the two microscopes was 99%. Between-test agreement for the FTA-ABS test with the conventional reporting system and the FTA-ABS DS test with the new reporting system was 95%. Sensitivity calculations based on reactivity for the 35 syphilis sera were 94% for the FTA-ABS DS test and 91% for the FTA-ABS test. Specificity calculations based on non-reactivity of nonsyphilis sera were 98% for the FTA-ABS DS test and 93% for the FTA-ABS test. Differences in percentages appeared to be related to borderline readings in the FTA-ABS test. For example, if the same reporting system was used for the reference FTA-ABS test, the specificity was 97%. When sera were examined within 48 h, no difference was observed in results obtained with heated and unheated sera. Sera frozen for 2 weeks showed comparable results in the FTA-ABS DS test and the FTA-ABS test. These findings strongly support the recommendation that the FTA-ABS DS test be accepted as a confirmatory test for syphilis. The new reporting system for the FTA-ABS DS test would be advantageous for the reference FTA-ABS procedure.

The fluorescent treponemal antibody absorption (FTA-ABS) double-staining (DS) test (2, 3, 5, 6) has been reported as an alternative to the conventional FTA-ABS test for detecting treponemal antibody when incident-light microscopy is used. This procedure incorporates a rhodamine-labeled, class-specific, anti-human immunoglobulin G primary stain and fluorescein-labeled anti-treponemal globulin as counterstain. In this evaluation, we obtained 346 fresh sera and investigated the sensitivity and the specificity of the FTA-ABS DS test and the conventional FTA-ABS test. Primary parameters for this investigation included two microscopists with two microscope models and the new FTA-ABS DS test reporting system (3). The effects of sera heated at 56°C versus unheated sera and freezer storage were examined for some of the sera.

MATERIALS AND METHODS

FTA-ABS test reagents and procedure. *Treponema pallidum* antigen, sorbent, control sera, and fluorescein-labeled anti-human globulin were reference reagents for the FTA-ABS test and were prepared by Biological Products Division at the Centers for Disease Control, Atlanta, Ga. The FTA-ABS test was performed according to the reference procedure in the *Manual of Tests for Syphilis* (7).

FTA-ABS DS test reagents and procedure. *T. pallidum* antigen and sorbent were the same as the reference reagents for the FTA-ABS test. Reactive and nonspecific control sera and the fluorescein-labeled anti-treponemal globulin for counterstaining were as previously prepared for the FTA-ABS DS test procedure (2). The primary stain, rhodamine-labeled $F(ab')_2$ fragment of goat anti-human immunoglobulin G, was obtained commercially from Kallestad Laboratories, Chaska, Minn. The FTA-ABS DS test procedure was as described by Hunter et al. (3). For the DS proce-

dure, slides with two 15-mm rings each were used (10-224; Cell-Line Associates, Inc., Newfield, N.J.).

Reporting system. A total of 346 fresh sera were tested in this evaluation, with at least one FTA-ABS DS test result and one conventional FTA-ABS test result obtained on each serum. For the FTA-ABS DS test, results were reported by both the conventional FTA-ABS test reporting system and the DS reporting system. The conventional reporting system was used for the reference FTA-ABS test results. The B-R1+ intensity-of-staining result is the essential difference between the systems. By the new DS system, a serum with either an R1+ initial result and a B or an N repeat result or an initial B result and a repeat R1+ result is reported as B. A serum with a B initial result and a B or N repeat result is reported as N, whereas in conventional FTA reporting, the report would be B. In the DS system, a B report indicates that one reading of the serum was R1+ and another reading(s) was lower.

Microscope equipment. One microscopist used a Leitz Ortholux II microscope equipped with transmitted and incident illumination. The dark-field condenser (immersion D1.20-1.40), 10× oculars, and 40× high-dry objective were used for reading reference FTA-ABS tests results. Fluorescein fluorescence was read with a KP490 interference filter and a K530 barrier filter. With incident illumination, a Leitz N filter cube was used for reading rhodamine fluorescence, and an H filter with an additional K480 edge filter was used for reading fluorescein fluorescence. 6.3× oculars and a 100×/1.25 oil objective were used for reading. The microscope was equipped with an HBO200 lamp.

The second microscopist used an American Optical Series 120 Microstar microscope to read FTA-ABS DS test results. This microscope was equipped for incident illumination with an HBO50 lamp. The filter system supplied for rhodamine excitation was the American Optical FluorCluster 2074, which included an exciter 546 filter, a 560 dichroic beam splitter, and a barrier OG590 filter. A 2075 FluorCluster was supplied for fluorescein excitation, the 2075 cluster consisting of an exciter fluorescein isothiocyanate filter, a 500 dichroic beam splitter, and a barrier OG515 filter. The American Optical Microstar was equipped with 6× oculars and a 100×/1.25 oil objective. The second reader used a Leitz SM-M microscope equipped with a HBO200 lamp and a dark-field condenser for reading FTA-ABS test slides. A BG12 exciter filter and an OG1 barrier filter were used for fluorescein fluorescence. The readings were made with 10× oculars and a 45× high-dry objective.

Sera. A total of 346 sera were obtained from patients visiting the DeKalb County Sexually Transmitted Disease Clinic, Atlanta, Ga. These sera included 35 from patients with clinically diagnosed syphilis. Initial examination was performed within 48 h of storage at 2 to 8°C. Repeat testing was performed within 2 weeks on serum samples stored at −20°C.

Evaluation. A total of 346 sera were examined. The FTA-ABS DS test was performed in two laboratories with readings made by two microscopists with the two microscopes described above. FTA-ABS DS test results were compared with FTA-ABS reference test results. The FTA-ABS test was read alternately by the microscopists on their respective microscopes. Of the 346 sera, 76 were read in the FTA-ABS DS test only once, on alternate microscopes, and data were included in an overall comparison of the 346 sera. Results were compared with those of the reference FTA-ABS test.

Heated versus unheated sera. A sample of each of 166 sera was tested fresh (within 48 h) in the FTA-ABS DS test without heating along with a sample of the same serum that had been heated at 56°C for 30 min. These two FTA-ABS DS test results were compared with the FTA-ABS test results obtained with heated sera. The sera were read alternately by the two readers on their respective microscopes.

Freezer storage. Thirty-two sera that exhibited some degree of staining in the initial testing were retested after storage at −20°C for at least 2 weeks. Of these sera, 26 were from syphilitic individuals, and 8 of the 26 were read as B or R1+. An equal number of sera, reactive and nonreactive, were also retested to double-blind the second examination of the above sera.

RESULTS

Table 1 shows a comparison of the results of 27 syphilitic sera and 243 nonsyphilitic sera, read twice in the FTA-ABS DS test by two microscopists using two models of microscopes, with FTA-ABS test results. Results are presented by both reporting methods for the FTA-ABS DS test and by the conventional method for the reference FTA-ABS test. For the FTA-ABS DS test, both microscopists reported 25 reactive sera of a total of 27 sera in the syphilitic group, resulting in 100% agreement between the two microscopes and the two microscopists. Nearly identical results were reported by both microscopists for the 243 sera in the nonsyphilitic group. Overall test agreement in the FTA-ABS DS test for the 270 sera was 99% for two FTA-ABS DS test results and 98% for FTA-ABS test results. For the 27 syphilitic sera, agreement among the three results was 93%.

Table 2 shows FTA-ABS DS and FTA-ABS test results for 346 sera, including 35 syphilitic and 311 nonsyphilitic sera. In the FTA-ABS DS test, 33 of 35 syphilitic sera were reactive, resulting in 94% sensitivity by both reporting systems. In the FTA-ABS test, 32 of 35 syphilitic sera were reactive, resulting in 91% sensitivity. One serum that was reactive in the FTA-ABS DS test was reported to be borderline in the FTA-ABS test. As B readings were not interpreted as R or N (7), sensitivities between the two tests were not the same.

Specificity was determined by nonreactivity in nonsyphilitic sera. With the FTA-ABS DS test, 300 of 311 nonsyphilitic sera were nonreactive by the conventional reporting method, resulting in a specificity of 96%, whereas 305 of 311 sera were nonreactive by the DS reporting method, resulting in a specificity of 98%. For the FTA-ABS test, 290 of 311 sera were nonreactive, resulting in a specificity of 93%. Discrepancies were due primarily to the reporting of borderline sera.

TABLE 1. Comparison of microscopes: two FTA-ABS DS test results versus FTA-ABS test results for 270 sera[a]

Serum category	No.	FTA-ABS DS test												FTA-ABS test with FTA reporting system		
		Leitz						AO								
		FTA			DS			FTA			DS					
		R	B	N	R	B	N	R	B	N	R	B	N	R	B	N
Primary syphilis	5	3	1	1	3		2	3	1	1	3		2	2	2	1
Secondary syphilis	7	7			7			7			7			7		
Late/latent syphilis	15	15			15			15			15			15		
Nonsyphilis	243	2	5	236	2		241	2	6	235	2	2	239	3	9	231

[a] R, Reactive; B, borderline; N, nonreactive. FTA and DS are reporting systems. Leitz and AO are microscopes. Numbers represent the numbers of sera.

Overall agreement for the total 346 sera for the FTA-ABS DS test and the FTA-ABS test was 93% by the conventional method and 90% by the DS method of reporting FTA-ABS DS test results. Agreement for the 35 syphilitic sera between the FTA-ABS DS test and the FTA-ABS test was 97% by the conventional method and 94% by the DS method of reporting FTA-ABS DS test results. When both tests were reported by the DS system, agreement among the 35 syphilitic sera was 94%. By the DS reporting system, the sensitivity for the FTA-ABS test was 91%, specificity was 97%, and agreement between the FTA-ABS test results and the FTA-ABS DS test results was 98% overall.

Of the sera, 166 were tested unheated in the FTA-ABS DS test and heated at 56°C for 30 min in both the FTA-ABS DS test and the FTA-ABS test. Results obtained when both reporting methods were used are shown in Table 3. For FTA-ABS DS test results, agreement between unheated and heated sera was 98% by conventional reporting and 99% by DS reporting. Agreement between FTA-ABS DS test results and reference FTA-ABS test results for unheated sera was 96% by conventional FTA-ABS DS reporting and 93% by DS reporting. A higher degree of nonspecificity was seen in the reference FTA-ABS test than in the FTA-ABS DS test when either heated or unheated sera were used. Agreement between the FTA-ABS DS test results and the FTA-ABS test results for heated sera was 95% by conventional reporting and 92% by DS reporting of FTA-ABS DS test results.

In addition, 64 of these sera were frozen for at least 2 weeks and then retested, after having been tested by both procedures. Results after freezing showed little difference from those of initial testing, with a slight drop in sensitivity seen in both the FTA-ABS DS test and the FTA-ABS test. As seen in other testing, there were more borderlines in the FTA-ABS test than in the FTA-ABS DS test. The loss of sensitivity occurred in two of the sera that had given varying results throughout this evaluation.

DISCUSSION

The data from this evaluation showed comparable results between the FTA-ABS DS test and the FTA-ABS test when two microscopists used two different microscope models. This observation suggests that microscopes with appropriate filters for the FTA-ABS DS test are available from at least two manufacturers; no microscope problems were encountered in this study.

The FTA-ABS DS test appears to be a reliable

TABLE 2. FTA-ABS DS test results versus FTA-ABS test results for 346 sera incorporating the conventional versus the DS reporting systems[a]

Serum category	No.	FTA-ABS DS test						FTA-ABS test with FTA reporting system		
		FTA			DS					
		R	B	N	R	B	N	R	B	N
Primary syphilis	7	5	1	1	5		2	4	2	1
Secondary syphilis	9	9			9			9		
Late/latent syphilis	19	19			19			19		
Nonsyphilis	311	4	7	300	4	2	305	7	14	290

[a] In the new double-staining system, sera with all B and N (no R1+) were reported as N. R, Reactive; B, borderline; N, nonreactive. FTA and DS are reporting systems. Numbers represent numbers of sera.

TABLE 3. FTA-ABS DS test results for 166 sera unheated and heated at 56°C[a]

Serum category	No.	No. of reactive sera in the FTA-ABS DS test with indicated reporting system												FTA-ABS test with heated sera		
		Heated sera						Unheated sera								
		FTA			DS			FTA			DS					
		R	B	N	R	B	N	R	B	N	R	B	N	R	B	N
Primary syphilis	4	3		1	3		1	3		1	3		1	3	1	
Secondary syphilis	5	5			5			5			5			5		
Late/latent syphilis	9	9			9			9			9			9		
Nonsyphilis	148		4	144			148		7	141		1	147	2	10	136

[a] R, Reactive; B, borderline; N, nonreactive.

alternative to the FTA-ABS test when incident-light microscopy is used. Sensitivity and specificity for the FTA-ABS DS test for this group of sera proved to be excellent. For all 346 sera tested, sensitivity, based on results from sera obtained from syphilitic individuals, was 94% for the FTA-ABS DS test, regardless of the reporting method used, as compared with 91% sensitivity for the FTA-ABS test. Specificity based on nonreactivity in the nonsyphilis group was 96% for the FTA-ABS DS test by conventional reporting and 98% by the DS reporting system, as compared with 93% for the FTA-ABS test. These findings are supported by the work of other investigators (2, 3, 5, 6).

The FTA-ABS DS test procedure was described to update the FTA-ABS test for new microscopes equipped with incident illumination. We found the technical performance of the test and the reading by incident illumination to be less time-consuming, more accurate, and easier on the eyes than the conventional FTA-ABS test method. The usefulness and reproducibility of the test are supported by the excellent correlation between the results obtained when two microscopists used two microscopes; results from sera obtained from syphilitic patients agreed 100%, and overall agreement was 99% by either reporting system. There seemed to be almost no difference in results of the FTA-ABS DS test, regardless of whether the sera were heated at 56°C, a finding similar to those of previous studies (1, 4) of other treponemal testing methods in which no loss in sensitivity was observed. There was more non-specificity, particularly in nonsyphilitic sera in the conventional FTA-ABS test, than in the FTA-ABS DS test when either heated or unheated sera were used, regardless of the reporting system employed. Borderlines in the FTA-ABS test tended to be eliminated in the FTA-ABS DS test by better focusing of treponemes, more distinct plus readings, and the new DS reporting system. However, specificity and sensitivity may also be enhanced by the incorporation of pure reagents, such as the $F(ab')_2$ conjugate, in the FTA-ABS DS test as opposed to the whole globulin conjugate used in the reference FTA-ABS test. Likewise, we found little difference in results when sera frozen for at least 2 weeks were retested. The differences that did occur caused a slight drop in sensitivity and appeared to be related to individual sera rather than directly attributable to freezing, since the same sera had shifted in degrees of fluorescence throughout the evaluation. The FTA-ABS DS test is recommended as an acceptable confirmatory test for syphilis, along with the FTA-ABS test. Differences between the two tests were not great and, on the basis of our findings, we recommend the DS reporting procedure for both tests. The new DS reporting system (3) appears to deal more effectively with the problem of reporting borderlines.

LITERATURE CITED

1. **Hunter, E. F., M. R. Adams, L. H. Orrison, B. J. Pender, and S. A. Larsen.** 1979. Problems affecting performance of the fluorescent treponemal antibody-absorption test for syphilis. J. Clin. Microbiol. **9**:163–166.
2. **Hunter, E. F., R. M. McKinney, S. E. Maddison, and D. D. Cruce.** 1979. Double-staining procedure for the fluorescent treponemal antibody absorption (FTA-ABS) test. Br. J. Vener. Dis. **55**:105–108.
3. **Hunter, E. F., B. J. Pender, E. J. Kennedy, D. E. Pettit, C. M. Schubert, J. C. Feeley, S. A. Larsen, and B. E. McGrew.** 1981. Fluorescent treponemal antibody-absorption double-staining procedure. J. Clin. Microbiol. **14**:184–188.
4. **Larsen, S. A., D. D. Cruce, C. E. Farshy, and J. C. Feeley.** 1978. Heated versus unheated sera in a microhemagglutination assay for antibodies to *Treponema pallidum*. J. Clin. Microbiol. **8**:468.
5. **Mote, P. T., E. F. Hunter, C. M. Schubert, and J. C. Feeley.** 1980. Further studies with the fluorescent treponemal antibody-absorption double-staining procedure. J. Clin. Microbiol. **12**:402–405.
6. **Riggsbee, J. H., and C. L. Lamke.** 1981. An evaluation of the double-staining procedure for the fluorescent treponemal antibody-absorption (FTA-ABS) test. Lab. Med. **12**:232–234.
7. **U.S. Department of Health, Education and Welfare.** 1969. Manual of tests for syphilis, Public Health Service publication no. 411. National Communicable Disease Center Venereal Disease Program, Atlanta, Ga.