Automated Fluorescent Treponemal Antibody Test:  
Instrument and Evaluation

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Two evaluations of the automated fluorescent treponemal antibody (AFTA) test for the serodiagnosis of syphilis are described. The results of AFTA and manually performed fluorescent treponemal antibody-absorption (FTA-ABS) tests were compared on serum samples from clinically defined donor groups, and the reproducibility of each procedure was studied. Significant improvement of AFTA test results was obtained in the most recent study after developmental modifications of the instrument and test technique. AFTA test agreement with both syphilis and nonsyphilis categories was considered good. With the increasing usage of the FTA-ABS test as an effective tool for the diagnosis of syphilis, successful automation of this procedure is particularly timely and significant.

The fluorescent treponemal antibody-absorption (FTA-ABS) test was first described in 1964 (4). Preliminary data indicated comparable specificity to the Treponema pallidum immobilization (TPI) test and somewhat greater sensitivity. Results of evaluations and experimental studies (2, 5) performed since the original report have given support to and increased confidence in the use of this confirmatory test for the detection of syphilis antibodies.

Usage of the FTA-ABS test is increasing. Many laboratories perform the test daily but limit testing to specimens meeting specific criteria, since one serologist can perform only 40 to 50 tests per day. To increase availability of the FTA-ABS test to physicians, an automated procedure is desirable.

The Space Division, Aerojet-General Corp., in cooperation with the Venereal Disease Research Laboratory (VDRL), has developed equipment (SeroMatic System) to automate the FTA-ABS test (1). The equipment, in various stages of development, and the modified test technique were evaluated at VDRL and several other locations. Results of automated fluorescent treponemal antibody (AFTA) tests, with prototypes and original production model equipment, indicated the practicability of using the AFTA technique in the serodiagnosis of syphilis.

In an early evaluation of the first production model (evaluation I), agreement between the AFTA and FTA-ABS tests was 87.0% (G. W. Stout et al., Presented at Am. Pub. Health Ass. Annu. Meeting, Detroit, Mich., 1968). Because of the occurrence of random discrepancies, several changes were made on both the equipment and the AFTA test technique to correct the cause.

This paper describes the SeroMatic System and its use in two subsequent evaluations (II and III) of the provisional AFTA test (10). In evaluation II, AFTA and FTA-ABS tests were performed on 789 sera from clinically defined donor groups to study the agreement between the tests and the agreement of each test with clinical diagnosis; in addition, the reproducibility of both automated and manual procedures was determined. In evaluation III, 628 serum specimens submitted from ongoing clinical studies were tested with the automated test, using the improved instrument and technique, and with the manual test to study agreement between the two procedures and agreement of each procedure with clinical diagnosis.

MATERIALS AND METHODS

Equipment. The SeroMatic System for the AFTA test has been described in detail elsewhere (1). In brief, the system consists of a slide processor, reagent reservoirs, serum-sorbent blocks, and microscope attachments.

The slide processor is an electropneumatically controlled device with: (i) a circular rotating table, having 50 slide positions equally spaced around the periphery and advancing counter-clockwise 1/60th of a revolution per 1 min and 45 sec; (ii) a slide loading and unloading mechanism; (iii) a serum-sorbent applicator; (iv) two adjustable incubators having a combined capacity of 3 liters of distilled water and relative humidity of 95 to 100.0%; (v) a phosphate-buffered saline (PBS) wash station; (vi) two distilled-water wash stations; (vii) conjugate applicator, consisting of a Cornwall syringe (1-ml) and a diluted conjugate.
reservoir (10-ml capacity for 100 slides); and (viii) two slide-drying stations.

The plastic blocks for the serum-sorbent mixture have 10 specimen cavities and are reusable. The polyethylene reservoirs for water and PBS have capacities of 38 and 7.5 liters, respectively. The microscope attachment consists of a modified stage adapter and a pushbutton glycerol-feed assembly which maintains a glycerol coupling between the top of the condenser and the processed surface of the inverted slides. This procedure eliminates the manual procedure for mounting the specimens. Slides are sequentially injected and positioned on the stage of the microscope, requiring only a fine focus adjustment for reading.

A Leitz dark-field fluorescence microscope assembly equipped with an Osram HBO-200 mercury lamp, a Schott BG-12 exciter filter, and an OG-1 barrier filter was used for reading both automatically and manually processed slides at a magnification of 450 X. Fluorescence was measured subjectively by visual observation and graded numerically from nonreactive (−) to strongly reactive (+).

Reagents for FTA-ABS and AFTA tests. For the manual test, FTA-ABS test antigen, lyophilized T. pallidum Nichols strain, was prepared at VDRL and was used according to the published technique (9). For the automated test, the AFTA test antigen, prepared by the Space Division, Aerojet-General Corp. (6), is placed in the geometric center of a cover slip (22 by 40 mm) and mounted on a styrene plastic carrier (25 by 75 by 1 mm). The antigen slide (Redi-Fix) is then fixed in 10.0% methyl alcohol and stored at −20°C.

The same lyophilized fluorescein-labeled antihuman globulin (conjugate; 6, 7) and sorbent (8) prepared by Space Division, Aerojet-General Corp., were used in the FTA-ABS and AFTA tests. The conjugate titer for each method was determined independently. Other reagents used have the same specifications as published (9).

All reagents were pretested and found to be satisfactory for use in the FTA-ABS and AFTA tests.

Control sera. The same lyophilized control sera (reactive and nonspecific) prepared by the VDRL were used in both tests. Control sera and nonspecific staining controls were included at the beginning and end of each AFTA test run. A minimally reactive (1+) control was inserted after every 20 specimens as a reading standard.

Evaluation sera (II and III). Individual sera included samples from the VDRL serum bank, which had been stored at −20°C for various periods of time, and freshly collected sera. Donors were classified in the following clinical categories. The nonsyphilis category included (i) presumed normals (no history of past or present syphilitic or other treponemal infection, and nonreactive in the VDRL slide test); (ii) false-positive (FP) reactors, acute or chronic (reactive in the VDRL slide test and nonreactive in the TPI and prior FTA-200 or FTA-ABS tests, with no clinical or historical evidence of syphilis); and (iii) diseases other than syphilis (clinical patients with a variety of diseases with no clinical or historical evidence of syphilis). The syphilis category included treated and untreated documented syphilitics.

Specimens were obtained from patients admitted to venereal disease clinics, general outpatient clinics and hospitals, and those on whom routine blood tests were performed. The diagnosis of syphilis or nonsyphilis was made on the correlation of available historical, clinical, and serological data.

Specimens were processed in groups containing sera randomly selected from the various clinical categories. The serologist performing the tests did so without prior knowledge of the clinical category of the specimens.

Reproducibility study. In evaluation II, to determine test reproducibility within a laboratory, one serum from a patient diagnosed as late latent syphilis was divided into 120 samples; 60 samples were tested with each procedure. This serum was nonreactive in the VDRL slide test and reactive in prior TPI and FTA-ABS tests.

Test procedures. The FTA-ABS test was performed according to the published technique (9), and the AFTA test was performed according to the provisional technique, dated May 1969 (10). Manual operations performed in the AFTA test included processing of sera; measuring serum and sorbent into the numbered cavities of blocks; loading the instrument with the blocks, prenumbered antigen slides, and other reagents; and reading the processed test slides. All other steps of the indirect fluorescent-antibody test were performed automatically by the slide processor.

RESULTS

The reactivity rates of the FTA-ABS and AFTA tests with clinical categories are shown in Table 1. Reactivity of the combined syphilis sera was 92.5% with the FTA-ABS test and 90.6% with the AFTA test in evaluation II and 86.5% FTA-ABS and 83.5% AFTA in evaluation III. Two-thirds of the syphilis specimens included in evaluation III were obtained from patients adequately treated for early syphilis. Fifteen of these sera were nonreactive and 15 were borderline. This accounts for the apparent reduction in sensitivity.

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Total sera tested</th>
<th>FTA-ABS</th>
<th>AFTA</th>
<th>FTA-ABS</th>
<th>AFTA</th>
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<tr>
<td>Syphilis, treated and untreated</td>
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<td>Evaluation II</td>
<td>427</td>
<td>92.5</td>
<td>90.6</td>
<td>3.5</td>
<td>2.8</td>
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<td>86.5</td>
<td>83.5</td>
<td>3.8</td>
<td>5.5</td>
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<td>Nonsyphilis</td>
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<td></td>
<td></td>
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<td>Evaluation II</td>
<td>362</td>
<td>1.1</td>
<td>7.2</td>
<td>2.8</td>
<td>2.2</td>
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<tr>
<td>Evaluation III</td>
<td>446</td>
<td>0.4</td>
<td>1.8</td>
<td>1.1</td>
<td>2.4</td>
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</table>

TABLE 1. Reactivity of FTA-ABS and AFTA tests on syphilis and nonsyphilis sera
of both tests. The reactivity obtained in these studies on syphilis categories is within the range obtained in other studies on the FTA-ABS test (5). In the nonsyphilis category, more false-positives were obtained with the AFTA test than with the FTA-ABS test during evaluation II (7.2% AFTA and 1.1% FTA-ABS); however, improvement was seen in evaluation III (1.8% AFTA and 0.4% FTA-ABS).

Agreement, partial agreement, and disagreement of results of the two tests in both evaluations are shown in Table 2. Those specimens in complete agreement were reactive, border, or non-reactive in both tests; partial agreement between the two tests included a reactive versus borderline or a borderline versus nonreactive report. Results of the two tests agreed 89.6% in evaluation II and 94.9% in evaluation III. Disagreement occurred in 5.7% of all tests in evaluation II and 0.8% in evaluation III.

Table 3 shows the agreement of FTA-ABS and AFTA tests with the clinical diagnosis in evaluations II and III. Percentage agreement was calculated from the number of tests in complete agreement plus one-half the number in partial agreement, divided by the total number of tests.

Results indicated a significant improvement in the AFTA test with nonsyphilis specimens between the two evaluations. Evaluation II resulted in 91.7% AFTA agreement with nonsyphilis specimens, whereas evaluation III showed 96.9%.

Reproducibility of the serum tested 60 times with each test in evaluation II is shown in Table 4. The FTA-ABS test was minimally reactive (1+) one time, the other results being 2+ or greater; the AFTA test gave one borderline and three nonreactive results in addition to 56 readings of 2+ or greater.

**DISCUSSION**

Early experience with the SeroMatic System demonstrated its potential to process 150 to 200 test slides per day, which could be read as tests were completed. The slide processor automatically advances the special antigen slides through a series of operations which include addition of specimen and reagents, incubation periods, and washing and drying of the finished slides. The microscope attachment can be adapted to virtually any microscope, simplifies the handling of slides, and greatly facilitates reading of slides. With this system, a serologist can perform many more fluorescent-antibody tests per day than can be done by the manual method.

After completion of each of several studies on the AFTA test, both at the VDRL and in one state public health laboratory, results were analyzed and the instrument and test technique were modified to increase test sensitivity and to reduce the number of incorrect results. The lower sensitivity of the AFTA test on syphilis category specimens and random disagreements with the FTA-ABS test results in all specimen categories seemed more related to malfunctions of the instrument than to operator error. Various steps of the test technique were revised, and certain ancillary reagents were modified. This resulted in the

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**TABLE 2.** Per cent agreement of FTA-ABS and AFTA test results in evaluations II and III

<table>
<thead>
<tr>
<th>Determination</th>
<th>Evaluation II</th>
<th>Evaluation III</th>
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<tr>
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<td>No.</td>
<td>Per cent</td>
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<tr>
<td>----------------</td>
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<tr>
<td>Total sera tested</td>
<td>789</td>
<td>100.0</td>
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<tr>
<td>Agreement (R-R, B-B, N-N)*</td>
<td>707</td>
<td>89.6</td>
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<td>Partial agreement (R-B, B-N)</td>
<td>37</td>
<td>4.7</td>
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<tr>
<td>Disagreement (R-N, N-R)</td>
<td>45</td>
<td>5.5</td>
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</table>

* R, reactive; B, borderline; N, nonreactive.

**TABLE 3. Agreement of AFTA and FTA-ABS tests with clinical diagnoses**

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Total sera tested</th>
<th>Per cent agreement with clinical diagnoses*</th>
<th>FTA-ABS</th>
<th>AFTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis, treated and untreated</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluation II</td>
<td>427</td>
<td>94.3</td>
<td>92.0</td>
<td></td>
</tr>
<tr>
<td>Evaluation III</td>
<td>182</td>
<td>88.2</td>
<td>86.3</td>
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<tr>
<td>Nonsyphilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluation II</td>
<td>362</td>
<td>97.5</td>
<td>91.7</td>
<td></td>
</tr>
<tr>
<td>Evaluation III</td>
<td>446</td>
<td>98.9</td>
<td>96.9</td>
<td></td>
</tr>
</tbody>
</table>

* Per cent agreement = (complete agreement + 0.5 partial agreement)/(total number specimens) × 100.
provisional technique used in these evaluations. During these studies, personnel from Aerojet-General made several adjustments and modifications on the slide processor. These included redesigning the incubators to maintain a constant temperature and to decrease the reagents drying on the slides, improving the mechanism for delivering serum-sorbent mixture to the slides so that the entire specimen is applied to the correct slide and splatter of specimens to the adjacent slides is eliminated, and redesigning the serum-sorbent blocks for more efficient cleaning and prevention of specimen leakage. During evaluation II, the instrument was monitored continuously, and minor malfunctions which occurred were corrected manually. Because of the improved operation of the instrument, monitoring was done only on a spot-check basis in evaluation III.

A comparison of results obtained in the preliminary study (G. W. Stout et al., presented at Am. Pub. Health Ass. Annu. Meeting, Detroit, Mich., 1968) and in evaluation. II and III indicates that changes in the instrument and test technique have significantly improved agreement of AFTA test results with clinical diagnosis. FTA-ABS test results, however, were slightly more sensitive, specific, and reproducible.

Reproducibility of both procedures was studied in evaluation II by testing 60 replicate samples of a single reactive syphilis serum (Table 4). Although there was a range in plus readings with the FTA-ABS test, the reportable test results were reactive for all samples. With the AFTA test, one sample would have been reported borderline and three nonreactive.

Borderline results occurred with both syphilis and nonsyphilis specimens, a somewhat higher proportion occurring in the syphilis categories with both tests. It has been reported (3) that borderline results with the FTA-ABS test cannot be interpreted as reactive or nonreactive because of the problem of reproducibility in this area. The distribution of borderline results obtained with the AFTA test in these evaluations also indicates that a diagnosis of syphilis or nonsyphilis cannot be made on borderline results.

In addition to the VDRL, three state public health laboratories are conducting evaluations, one of which has been completed (Coffey et al., unpublished data). As with the FTA-ABS and other new test procedures, performance improved as the serologists gained experience and proficiency.

Increasing usage and reliance upon the FTA-ABS test for the prompt resolution of problem cases, as well as on all reactive specimens, by public health and clinical laboratories suggest that the successful automation of this procedure is timely and significant and could be an effective tool in the serodiagnosis of syphilis.

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LITERATURE CITED