Further Evaluation of the Automated Fluorescent Treponemal Antibody Test for Syphilis

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Improvements in the equipment for the automated fluorescent treponemal antibody (AFTA) test for syphilis prompted this comparative study of the AFTA and its manual counterpart, the fluorescent treponemal antibody-absorption (FTA-ABS) test. The AFTA equipment operated satisfactorily, required only minimal monitoring, and afforded a three-to-four fold increase over the number of sera that could be tested manually by one serologist. The AFTA and FTA-ABS tests agreed well with only 2.1% of the sera yielding conflicting results. The AFTA was less precise than the FTA-ABS on sera retested because of original conflicting results and on sera retested within the same run to determine reproducibility. However, these differences were not large, and AFTA test performance was considered to be within the limits acceptable for a diagnostic serological procedure.

The SeroMatic System developed by Aerojet-General Corp. (1) provides a tool for doing large numbers of indirect fluorescent antibody tests with a considerable saving in staff time over that required for manual methods. This system adapted for the automated fluorescent treponemal antibody (AFTA) test was evaluated at the Venereal Disease Research Laboratory (3), in this laboratory (2), and in the health department laboratories of two other states. Mechanical problems encountered in these studies led to modifications of the equipment. The purpose of the present study was to evaluate the modified equipment by comparing its performance in the AFTA test with that of the manual fluorescent treponemal antibody-absorption (FTA-ABS) test on sera submitted to this laboratory for FTA-ABS testing.

MATERIALS AND METHODS

Test procedure. The provisional technique for the AFTA test was used (5). The FTA-ABS procedure developed at the Venereal Disease Research Laboratory (4) was employed, except that slides were prepared on the day of use and fixed in 10% methanol for 5 min (6).

Reagents. Reagents for the AFTA test were generously supplied by the Aerojet-General Corp. Antigen for the FTA-ABS test was prepared in this laboratory (6); other reagents were obtained from commercial sources.

Equipment. The AFTA test employed the SeroMatic System (R) comprising an electropneumatically controlled slide processor and a microscope stage attachment (1).

Slides processed for either procedure were examined with a Zeiss fluorescence assembly with an Osram HBO200 mercury lamp in combination with a Zeiss microscope equipped with a dark-field condenser, a BG-12 exciter filter, and a no. 50 barrier filter.

Test sera. Five hundred and twenty-one individual human sera were tested independently by both the AFTA and FTA-ABS methods. The only criteria for selection of specimens were that they had been submitted for FTA-ABS testing and possessed sufficient volume to be tested by both procedures. Sera were stored at 6 C until testing was completed.

Reproducibility sera. As a measure of within run reproducibility, 49 sera were tested in duplicate by the AFTA procedure and 41 sera in duplicate by the FTA-ABS test.

Control sera. Control sera for the AFTA test were obtained from the manufacturer. FTA-ABS control sera were prepared in this laboratory.

RESULTS

The reactions obtained in the AFTA and FTA-ABS tests are given in Table 1; the AFTA test yielded five more reactive and eight more borderline results than were found in the FTA-ABS test. A comparison of the reactivity of the two tests was carried out. The AFTA and FTA-ABS tests agreed, giving the same reactions in 456 (87.5%) of the 521 sera tested (227, reactive; 4, borderline; 225 nonreactive). Partial agreement was obtained in an additional 54 sera (10.4%) in which 14 of these sera were reactive (AFTA, 11; FTA-ABS, 3) in one test and borderline (AFTA, 3; FTA-ABS, 11) in the other, and 40 were nonreactive (AFTA, 12 nonreactive; FTA-ABS, 28 nonreactive).

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tive) in one test and borderline in the other (AFTA, 28; FTA-ABS, 12). Complete disagree-
mement between the results of the AFTA and FTA-
ABS tests was found in 11 sera (2.1%); 4 were
reactive only in the AFTA test and 7 only in the
FTA-ABS test. Only 6 of the 11 specimens show-
ing complete disagreement contained sufficient
serum volume to repeat both the AFTA and
FTA-ABS tests; these results are shown in Table
2. When repeat testing verified the original result
of one test, but not the other, the unverified origi-
nal test result was considered to have been in
error. There were three such errors in the AFTA
test and one in the FTA-ABS test. Disagreements
between the two tests persisted upon retesting the
remaining two specimens.

As a measure of test reproducibility, approxi-
mately every 11th specimen was retested by in-
serting it at random within the next set of 10
specimens. The differences between the duplicate
tests are shown in Table 3. In the AFTA test, 59%
of the sera tested in duplicate gave the same plus
reading, 96% agreed within ± 1 plus, and no differences were greater than 2 plus. In the FTA-
ABS, 80% of the duplicate tests gave the same
reading and all agreed within ± 1 plus. It is pos-
sible that reader bias may have influenced the
reproducibility of the FTA-ABS test more than
the AFTA. In the AFTA test the reader was not
aware that a serum was being retested to deter-
mine reproducibility until after the slide was read.
In the FTA-ABS test, although the reader scrupu-
lously avoided examining the previous result, he
was aware at the time the slide was read that the
serum was being retested to determine repro-
ducibility.

**DISCUSSION**

The AFTA test, by automating many of the
time-consuming aspects of the manual FTA-ABS
test, provides a tool for substantially increasing
the number of sera that can be tested by one
serologist. As part of a national field trial, the
AFTA test had previously been evaluated in this
laboratory by comparing its performance with
that of the FTA-ABS test on sera from clinically
defined donor groups (2). Although the results
of the two tests disagreed in 3.5% of the sera
tested, the majority of these differences were not
true, repeatable differences between the two tests
but were apparently due to test error, with 2.7% of
the sera yielding such errors in the AFTA test
and 0.6% in the FTA-ABS test. These results
were achieved with continuous monitoring of the
AFTA slide processor and with manual correc-
tion of observed mechanical errors. Since that
study was completed, changes have been made in
the slide processing equipment to improve test
performance and eliminate the need for constant
monitoring. Lewis and his associates at the
Venereal Disease Research Laboratory reported
that changes in the equipment had substantially
improved the accuracy of the AFTA test (3).

The study reported here was undertaken to
evaluate the improved equipment on sera sub-

<table>
<thead>
<tr>
<th>Test</th>
<th>Total sera</th>
<th>Reactive</th>
<th>Borderline</th>
<th>Nonreactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFTA</td>
<td>521</td>
<td>242</td>
<td>35</td>
<td>244</td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>521</td>
<td>237</td>
<td>27</td>
<td>257</td>
</tr>
</tbody>
</table>

**Table 1. Reactivity of AFTA and FTA-ABS tests**

<table>
<thead>
<tr>
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<td>FTA-ABS</td>
<td>521</td>
<td>237</td>
<td>27</td>
<td>257</td>
</tr>
</tbody>
</table>

**Table 2. Original and repeat test results on sera giving conflicting results in the AFTA and FTA-ABS tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>AFTA run no.</th>
<th>Serum no.</th>
<th>Original test results&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Repeat test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original AFTA result not verified by repeat test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0313</td>
<td>R (2+)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>IV</td>
<td>1395</td>
<td>N</td>
<td>R (4+)</td>
<td>R (4+)</td>
</tr>
<tr>
<td>IV</td>
<td>1845</td>
<td>N</td>
<td>R (4+)</td>
<td>R (3+)</td>
</tr>
<tr>
<td>Original FTA-ABS result not verified by repeat test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4752</td>
<td>R (3+)</td>
<td>N</td>
<td>R (2+)</td>
</tr>
<tr>
<td>Both results verified by repeat test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>3122</td>
<td>N</td>
<td>R (1+)</td>
<td>N</td>
</tr>
<tr>
<td>VII</td>
<td>3231</td>
<td>R (3+)</td>
<td>N</td>
<td>R (2+)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: R, reactive; N, nonreactive.
The automated equipment operated well, required only minimal monitoring, and yielded more satisfactory results than in the previous study. Although exact agreement between the AFTA and FTA-ABS tests was obtained in only 87.5% of the 521 sera tested, an additional 10.4% gave borderline reactions (6% in the AFTA and 4.4% in the FTA-ABS). The two tests disagreed, one testing being reactive and the other nonreactive, in only 2.1% of the sera. Repeat testing by both the manual and automated procedures of 6 of these 11 sera verified the original FTA-ABS result in 5 sera and the original AFTA result in 3 sera. The automated test was less precise than the manual procedure in duplicate tests done to measure reproducibility. However, these differences were not large, and, in our opinion, the performance of the AFTA test in this study was within the limits acceptable for a diagnostic serological procedure.

![Table 3. Within-run reproducibility of AFTA and FTA-ABS tests](image)

The serologists doing the test preferred the automated to the manual procedure because of the ease of processing slides and especially because of the facilitated reading. We estimate that a three- to fourfold increase over the number of sera tested manually by one serologist can be achieved with the automated equipment. We would recommend that individuals planning to substitute the AFTA for the FTA-ABS test first undertake parallel testing with both procedures on sera representative of the type to be tested. This will establish the relative precision of the two procedures for that laboratory and provide information necessary for intralaboratory control and for interpretation of AFTA test results. The results of the comparative study reported here substantiate the conclusion of Lewis and his associates (3) that the AFTA test can now provide an effective tool for serological testing.

### LITERATURE CITED


