Evaluation of an Enzyme-Linked Immunosorbent Assay for Treponemal Antibody

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A new enzyme-linked immunosorbent assay with Treponema pallidum antigen bound to ferrous metal beads (Syphilis Bio-EnzaBead; Litton Bionetics Laboratory Products) was compared with the standard fluorescent treponemal antibody-absorption test for syphilis. Bio-EnzaBead and fluorescent treponemal antibody-absorption tests were done on 218 specimens from documented cases of syphilis, on 315 sera from individuals with diseases other than syphilis, and on sera submitted to a public health laboratory for premarital (304 specimens) or diagnostic (501 specimens) tests for syphilis. Agreement between the Bio-EnzaBead and reference tests ranged from 93.0% for sera for the diagnostic test to 99.5% for sera from patients with syphilis. The overall agreement among the 1,338 sera tested was 96.3%. The reproducibility of the Bio-EnzaBead test with 60 coded sera of graded reactivity was 97%. The test is easy to perform, the indicator results are clear and unequivocal, and the findings are comparable to those of the fluorescent treponemal antibody-absorption test.

The serodiagnosis of syphilis and other treponematoses depends to a large extent on the results of a non-BP treponemal reagin test, supplemented when indicated by a confirmatory treponemal antibody test (1, 3, 5). Tests for treponemal antibody currently recognized as standard procedures are the fluorescent treponemal antibody-absorption (FTA-ABS) and FTA-ABS double-staining procedures and the microhemagglutination assay for Treponema pallidum antibodies and hemagglutination treponemal test for syphilis (2, 4, 8, 15).

A new test, an enzyme-linked immunosorbent assay (ELISA) for treponemal antibody, has now been developed by Litton Bionetics Laboratory Products, Charleston, S.C. The technique, called Syphilis Bio-EnzaBead, has recently been recognized by the Centers for Disease Control as a provisional treponemal test for syphilis (Reagents Evaluation Program Supplement no. 28, 1984). The test involves the use of Treponema pallidum (Nichols strain) organisms fixed on ferrous metal beads as solid-phase antigen, peroxidase-conjugated anti-human immunoglobulins as the second antibody, and 2,2'-azino-di-[3-ethyl-2,2'-dihydro-6-benzthiazolinesulfonate] (ABTS) as the enzyme substrate. Antigen and control beads are moved simultaneously into and out of serum specimens, test reagents, and washes by means of a magnetic transfer device. The presence of treponemal antibody in serum is indicated by an enzyme substrate color of intensity equal to or greater than a minimum (1+) reading standard.

To determine the effectiveness of the Bio-EnzaBead test in detecting treponemal antibody and to assess it as an alternative to the standard procedures, we examined 1,338 sera by the Bio-EnzaBead and standard FTA-ABS tests (14, 15). The new ELISA procedure is described, and the results are compared with the FTA-ABS findings in this report.

MATERIALS AND METHODS

Standard tests for syphilis. Venereal Disease Research Laboratory (VDRL), automated reagin (ART), and fluorescent treponemal antibody-absorption (FTA-ABS) tests were done by published procedures with commercial reagents listed by the Centers for Disease Control. FTA-ABS readings of 1+ fluorescence, confirmed by repeat testing, were recorded as reactive; those less than 1+ were recorded as nonreactive. Serum controls and reading standards from the Centers for Disease Control or from the Center for Laboratories and Research were included in all test runs.

ELISA procedure. Bio-EnzaBead tests were conducted as directed on the product insert with reagents provided by the manufacturer. In addition to the test kit, 96-well plastic plates and a magnetic transfer device, available from Litton Bionetics, and a 37°C incubator are required. Serum specimens are diluted 1:100 in phosphate-buffered saline containing normal goat serum and Tween 20 and dispensed in 0.2-ml amounts into test and control wells on the plate. Diluted negative, low-positive (1+), and high-positive (4+) human reference sera are included on each plate. Ferrous metal 4-mm beads coated with T. pallidum antigen and control beads coated with normal rabbit tissue extract are dispensed simultaneously into the test and control wells by means of a special magnetic transfer device. The plate is covered and held in an incubator for 90 min at 37°C. The beads are washed by magnetic transfer 12 times into and out of a wash plate containing 0.2 ml of water with Tween 20 and moved to a third plate containing 0.2 ml of the second antibody reagent, an optimum dilution of peroxidase-conjugated goat anti-human immunoglobulin. The plate is covered and incubated as before. The beads are washed 12 times and moved to a plate containing 0.2 ml of the substrate indicator ABTS. The plate is held for 10 min at room temperature. A 0.025-ml volume of a 1.25% sodium fluoride stop solution is added to all wells and gently swirled about them. The beads are removed with the transfer device and discarded. The plate is set on a white background, and the colors in the wells are compared with the low-positive (1+) and high-positive (4+) reference serum colors. The comparison is made immediately or within 2 h. The color intensity in the wells is scored from negative (negligible color) to 4+ (dark green). An intensity of 1+ or greater in a test well is reported as reactive; specimens showing definite color less than 1+ are

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TABLE 1. Bio-EnzaBead and FTA-ABS test results on 304 VDRL- and ART-nonreactive, presumed-normal sera and on 301 VDRL-reactive specimens taken for serodiagnostic tests for syphilis

<table>
<thead>
<tr>
<th>FTA-ABS result&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bio-EnzaBead result for:</th>
<th>Reactive serodiagnostic test sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonreactive-normal sera</td>
<td>Reactive</td>
</tr>
<tr>
<td>R</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>N</td>
<td>2</td>
<td>322</td>
</tr>
</tbody>
</table>

<sup>a</sup> R. Reactive; N. nonreactive.

retested. Retests read as 1+ or more are reported as reactive; others are reported as nonreactive. The test is unsatisfactory if control well color intensity equals or exceeds that of the test well.

Study protocol. The evaluation was conducted in accordance with guidelines established by the Centers for Disease Control (14). The guidelines describe the specimen categories and serum panels to be examined and the calculations to be made. Specimen categories are (i) documented syphilis, (ii) diagnostic problems, (iii) presumed normals, and (iv) disorders other than syphilis. Serum panels are multiple samples of graded reactivity. The sensitivity, specificity, and agreement for the tests compared and the reproducibility of the test being evaluated were calculated.

Specimens. Serum specimens from the Centers for Laboratories and Research and the Albany Medical Center Hospital were used. Samples were kept not more than 3 days at 5°C or were stored at −20 or −70°C before the tests. The following groups were tested: (i) sera from documented syphilis cases, comprising 218 VDRL- and ART-, and FTA-ABS-reactive sera from untreated primary (3 specimens), secondary (9 specimens), early latent (less than 1 year duration) (7 specimens), and late latent (5 specimens) disease, and from treated primary (62 specimens), secondary (45 specimens), and late latent (87 specimens) disease; (ii) specimens for serodiagnosis of syphilis, comprising 501 VDRL-reactive sera; (iii) presumed-normal sera, comprising 304 VDRL- and ART-nonreactive premarital samples; and (iv) sera from individuals with nontreponemal disorders, comprising 315 VDRL-nonreactive sera unequivocally reactive in serological tests for diseases other than syphilis (see Table 2). Group (i) sera had been tested by FTA-ABS and stored at −70°C. Other serum groups were examined by FTA-ABS on the day before, or on the day of, the Bio-EnzaBead tests. Tests were done on various sera from the specimen groups by different technicians who had no knowledge of the results of the other treponemal tests.

Reproducibility panel. A panel of 60 coded specimens derived from six serum pools of graded reactivity were examined. The specimens were tested at a rate of six each day for five consecutive test days, and six on one day during each of the five following test weeks.

RESULTS

The Bio-EnzaBead test was reactive with all but one of the 218 specimens from documented cases of syphilis. The nonreactive specimen was from a patient who had been treated for late latent disease. The indicator color of the first ELISA test of that serum was <1+; a second test, conducted simultaneously with a confirmatory FTA-ABS test, was also seen as <1+. The FTA-ABS test was read as 3+, and the specimen was therefore recorded as Bio-EnzaBead nonreactive, FTA-ABS reactive. No differences were observed in the extent of substrate color intensity among the untreated and treated or early and late disease categories. Negligible (<1+) color was seen in the control wells of three sera, but since the test well colors were judged to be greater than 1+ the sera were recorded as reactive.

The findings with presumed normal specimens are shown in Table 1. The results agreed for 299 of the 304 sera. The percent agreement was therefore 98.4%. The specificity of the Bio-EnzaBead test was 98% (298/304); that of the FTA-ABS test was 98.4% (299/304).

The Bio-EnzaBead and FTA-ABS test results on specimens submitted for the serodiagnosis of syphilis agreed for 466 of the 501 specimens (Table 1). The 93% agreement is the lowest found for any of the serum groups. Moreover, the number of Bio-EnzaBead and FTA-ABS minimum (1+) reactions and equivocal (<1+) findings that necessitated repeat testing were highest with this serum category.

Results of tests on specimens from diseases other than syphilis are shown in Table 2. There was agreement between the tests with 307 (97.5%) of the 315 sera. All of the serological test results are summarized in Table 3. Overall agreement between the two procedures was 96.5%.

The reproducibility of the Bio-EnzaBead test with the special coded sera is shown in Table 4. The test readings made during this study were exactly the same as those recorded for the parent serum pools in 37 of 60 trials (63.3%) and within one gradation of that expected in 58 of 60 trials (96.6%).

DISCUSSION

The excellent agreement between the Bio-EnzaBead and FTA-ABS results with specimens from confirmed cases of syphilis indicates that the Bio-EnzaBead test is as effective as the reference test in detecting treponemal antibody. The 99.5% overall sensitivity with sera from cases at all stages of syphilis compares favorably with values of 89.3 and 99.0% reported for conventional ELISAs (12, 16). Furthermore, the Bio-EnzaBead test was reactive with all 65 primary and 86 of 87 late latent sera. The test therefore detects early as
TABLE 4. Reproducibility of Bio-EnzaBead readings of 60 coded sera examined at a rate of six each test day for 10 days

<table>
<thead>
<tr>
<th>Serum pool* (no. of trials)</th>
<th>Reading expectedb</th>
<th>No. of samples with reading ofc</th>
<th>4+</th>
<th>3+</th>
<th>2+</th>
<th>1+</th>
<th>+/-</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (6)</td>
<td>4+</td>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B (8)</td>
<td>2+</td>
<td></td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C (8)</td>
<td>3+</td>
<td></td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>D (16)</td>
<td>1+</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E (12)</td>
<td>N</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>F (10)</td>
<td>N</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

* Code made available at the conclusion of the trials.

b N. Negative.

c Test.

well as late antibody. Whether the earliest antibody would be detected was of course not conclusively established.

The probability that the Bio-EnzaBead test will be non-reactive with sera from people who do not have the disease (Table 1) is 98% (298/304). The specificity is essentially the same as that of the FTA-ABS test: 98.4% (299/304). As with the sensitivity data, findings may be compared with those reported for the conventional ELISA. The specificity for both an ELISA and an FTA-ABS test with a normal serum population like that examined in this study (12) was 98.5% (177/178). With a less well defined population (16), the specificity of an ELISA was 98.4% (258/260). The comparisons confirm that Bio-EnzaBead specificity is comparable to that of conventional ELISAs as well as to that of the FTA-ABS test.

It is difficult to evaluate the findings obtained with the sera submitted for diagnostic tests because the specimens do represent diagnostic problems (5, 11). It is likely, however, that the smallest amount of antibody present in some sera was at or near the minimum amount detectable by either or both of the tests. A greater proportion of discrepancies therefore occurred (6.9% of the problem sera examined) than would be expected (or were found) with normal sera containing no antibody (1.6% discrepancies) or with sera containing measurable amounts of antibody from individuals with syphilis (0.5%).

The Bio-EnzaBead test was reactive with 14 specimens in the category of diseases other than syphilis (4.4% of the 315 tested). This apparent false-positive finding is comparable to the 10 (3.2%) reactive with the FTA-ABS test. The greatest number of reactive sera, nine Bio-EnzaBead and seven FTA-ABS, were found in the antinuclear antibody (ANA) serum group. The disproportionate number of reactive ANA sera may be due in part to nucleoprotein on the surface of the T. pallidum antigen (7, 9, 10). No evidence of this phenomenon was detected, however. That is, no enzyme substrate color in the Bio-EnzaBead control wells or atypical ("beaded") fluorescence in the FTA-ABS test was seen with any of the specimens.

The 96.5% overall agreement between the Bio-EnzaBead and FTA-ABS tests is comparable to that found in comparisons of the standard hemagglutination and the FTA-ABS tests. Recent evaluations of hemagglutination tests show 93.7 to 93.3% agreement with the FTA-ABS test (4, 17).

The excellent reproducibility of the Bio-EnzaBead technique may be attributed to the use of beads for the solid-phase immunochemistry and to the efficiency with which the beads are manipulated (13). Factors that contribute to the uniformity and precision of the test process include (i) the relatively large and regular surface area of the beads, which provides for greater and more consistent exposure to antigen that is obtainable with conventional antigen-coated tubes or wells; (ii) the simultaneous movement of beads so that reaction, wash, and stop times are identical for all specimens, in contrast to the serial addition of reagents made in more typical assays; and (iii) the abrupt removal of beads and hence enzyme complexes from the final indicator solution so that there is a more definitive interruption of substrate color development than may be achieved by the addition of an enzyme stop only.

The Bio-EnzaBead test is easy to perform. No special training, microscopy, or other instrumental skills are needed. The reagents are stable for 28 days after opening. Any number (up to 90) of specimens may be processed by a technician in one test day.

In conclusion, the findings recommend consideration of the Syphilis Bio-EnzaBead test as an alternative to the FTA-ABS test.

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LITERATURE CITED

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