Evaluation of Commercial Serodiagnostic Kits for Toxoplasmosis

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Received 27 April 1987/Accepted 25 August 1987

The Centers for Disease Control (CDC) receives many requests for information about the usefulness of commercially available kits for the detection of antibodies to Toxoplasma gondii. Although the Food and Drug Administration has established packaging and labeling specifications to which such commercial products must conform when initially approved for marketing in the United States (4), no postmarketing requirements exist to ensure the continued quality of the kits. Many evaluations of one to three kits have been reported (1-3, 6-9, 12, 13), but results obtained in different laboratories are difficult to compare. We report here the results of a comparison of the sensitivity, specificity, and reactivity of seven nonimmunofluorescence commercial kits available in the United States for the serologic diagnosis of toxoplasmosis by the indirect immunofluorescence (IIF) test.

MATERIALS AND METHODS

Kits. We bought the following commercial kits on the U.S. open market: Cordia-T (lots 50502 and 50703; Diamedix Corp., Miami, Fla.), Toxo Bio-EnzaBead (lots 02523 and 02057; Litton Bionetics, Inc., Laboratory Product Div., Charleston, S.C.), Eiken Toxotest-MT (lot 2Z005; Syn-Kit Inc., Chatsworth, Calif.), TPM-Test (lots 3C7254A and 3E7249; Wampole Laboratories, Div. of Carter-Wallace, Inc., Cranbury, N.J.), ToxHAtest (lot K6812; Wellcome Reagents Div., Burroughs Wellcome Co., Research Triangle Park, N.C.), Toxoelisa (lots 11064 and 11007; Whittaker M.A. Bioproducts, Walkersville, Md.), and Toxoplasma-G FIAX (lots 3178059 and 3258119; Whittaker M.A. Bioproducts). Cordia-T, Toxo Bio-EnzaBead, and Toxoelisa were enzyme immunoassays (EIAs). Eiken Toxotest-MT was a latex agglutination (LA) test. TPM-Test and ToxHAtest were indirect hemagglutination (IHA) tests. Toxoplasma-G FIAX was a fluoroimmunoassay. Additional TPM-Test kits were supplied by Wampole Laboratories. All kits were used according to the instructions of the manufacturers. Serum controls supplied with the kits were included in each test.

Serum specimens. A battery of 100 serum specimens with various Toxoplasma antibody titers, as determined by the IIF test, was randomly assorted and coded. Each sample was tested 3 times by the IIF test; the mean of the three titers was used as a standard. The following are the number of serum samples at each IIF titer: 27, <1:16; 1, 1:16; 15, 1:64; 7, 1:128; 14, 1:256; 8, 1:512; 8, 1:1,024; 5, 1:2,048; 6, 1:4,096; 3, 1:8,192; 6, 1:16,384. All serum samples were tested once with each kit; those with grossly discrepant results compared with IIF results (i.e., negative result by IIF and positive result with the kit, positive result by IIF and negative result with the kit, or widely divergent titers) were retested.

For this study, true positive results were defined as those serum specimens that reacted to titers of ≥1:16 in the CDC IIF test. Kit sensitivity was thus the percentage of specimens positive by IIF that were detected as positive by the kit. Kit specificity was the percentage of specimens that gave titers of <1:16 by the IIF test and that also gave negative results with the kit.

CDC IIF test. The CDC IIF test for Toxoplasma antibodies was performed as described previously (11) by using reagents prepared at CDC. Tachyzoites recovered from mouse peritoneal fluid were fixed in 1% Formalin–phosphate-buffered saline for 30 min, washed in phosphate-buffered saline, adjusted to the appropriate dilution, dropped onto 12-well microscope slides, and air-dried. A fluorescein isothiocyanate-labeled goat immunoglobulin G (IgG) anti-human IgG conjugate was used at a 1:500 dilution with Evans blue counterstain, which was diluted 1:10,000. The tests were read on a microscope (American Optical Corp., Buffalo, N.Y.) equipped with epi-illumination (HBO-50 lamp), a binocular head, a ×40 dry objective, and a filter system (2072 Fluor Cluster). Serum samples were tested in twofold dilutions on 3 different days.
TABLE 1. Sensitivity and specificity of commercial kits for Toxoplasma antibody based on IIF results with 100 human serum samples

<table>
<thead>
<tr>
<th>Kit</th>
<th>No. (% agreement) negative (n = 27; IIF titer, &lt;16)</th>
<th>No. (% agreement) positive (n = 73; IIF titer, ≥16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma-G FIAX (&lt;16)</td>
<td>27 (100)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Cordia-T (&lt;30)</td>
<td>23 (85.2)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Toxo Bio-EnzaBead (&lt;100)</td>
<td>27 (100)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Toxoeisla (&lt;64.2)</td>
<td>21 (77.8)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>TPM-Test (&lt;64)</td>
<td>27 (100)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>ToxHAtest (&lt;64)</td>
<td>NR(^d)</td>
<td>NR</td>
</tr>
<tr>
<td>Eiken Toxotest-MT (&lt;16)</td>
<td>27 (100)</td>
<td>73 (100)</td>
</tr>
</tbody>
</table>

\(^a\) A total of 27 serum samples were negative by IIF, and 73 were positive by IIF.
\(^b\) The value for negative given by the manufacturers is given in parentheses.
\(^c\) Number of serum samples that agreed.
\(^d\) NR, Not readable.

RESULTS

Data on sensitivity and specificity for the kits are presented in Table 1. Qualitatively, results obtained with the Toxoplasma-G FIAX, Toxo Bio-EnzaBead, TPM-Test, and Eiken Toxotest-MT kits agreed 100% with those of the IIF test. Results of the Cordia-T test differed from those of IIF for four specimens (all false positives) and the Toxoelisa for six specimens (also all false positives). Serum samples that were negative by IIF but positive by EIA were tested in the Sabin-Feldman methylene blue dye test and were found to be negative by J. S. Remington (Palo Alto Medical Research Foundation, Palo Alto, Calif.).

The ToxHAtest kit (Burroughs Wellcome) did not give satisfactory results. The battery of sera was tested twice with the kit; on both occasions the reactions were unreadable on the day of testing. When the plates were held overnight and the cells were scrutinized very carefully, positive and negative reactions could be distinguished but reactivity was greatly reduced.

Quantitatively, the kits were difficult to compare because of the lack of standardization in the expression of results. When kit results were compared with the IIF results for serum samples with titers of ≥16, Spearman rank correlation coefficients were as follows: Toxoplasma-G FIAX, 0.9168; Eiken Toxotest-MT, 0.8293; Toxo Bio-EnzaBead, 0.7553; TPM-Test, 0.7206. Thus, titers obtained by the Toxoplasma-G FIAX test agreed most closely with those obtained by IIF.

Geometric mean titers were calculated for all specimens at each IIF titer (Fig. 1). The best agreement was usually found in the IIF titer range of 128 to 1,024. Mean titers determined with the Toxoplasma-G FIAX, Toxo Bio-EnzaBead, and TPM-Test kits were higher than those determined by IIF at a titer of 64, and lower titers were determined by all tests than those determined by IIF at an IIF titer of ≥4,096.

Cordia-T kit results are reported in international units, as defined by the World Health Organization. When IIF antibody titers were converted to international units and the results compared, serum samples with less than 250 IU consistently had higher titers by the Cordia-T test than by the IIF test, while those with more than 250 IU had lower titers (Fig. 2).

In the Toxoelisa kit absorbance ranges were used for the classification of results. The six specimens that were negative by IIF but positive by Toxoelisa reacted at values in the low and middle ranges (Table 2). Almost all specimens that had titers of ≥64, as determined by IIF, gave very positive reactions with the Toxoelisa kit.

FIG. 1. Comparison of titers obtained with the Toxoplasma-G FIAX, (IDT FIAX), Eiken Toxotest-MT (Eiken LA), Toxo Bio-EnzaBead (Liton IFA), and TPM-Test (Wampole IHA) kits with the CDC IIF test for Toxoplasma antibodies.

DISCUSSION

Each manufacturer included in the kit brochure a discussion of the limitations of the kit, such as false-positive reactions with rheumatoid factor-positive serum samples in the LA test kit or the lack of sensitivity in serum samples from patients with early acute infections in the IFA test kits. The battery of serum specimens used for this study did not.

FIG. 2. Comparison (in international units) of the Cordia-T kit and the CDC IIF test results for Toxoplasma antibodies.
TABLE 2. Comparison of results of the Toxoelisa kit and the IIF test for the detection of Toxoplasma antibodies

<table>
<thead>
<tr>
<th>Toxoelisa absorbance values (kit interpretation)</th>
<th>No. of serum samples with IIF titer of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.18 (negative)</td>
<td>&lt;16 16 32 64 128 ≥256</td>
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<tr>
<td>0.19-0.20 (equivocal)</td>
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</tr>
<tr>
<td>0.21-0.33 (low positive)</td>
<td></td>
</tr>
<tr>
<td>0.34-0.64 (mid-positive)</td>
<td></td>
</tr>
<tr>
<td>≥0.65 (high positive)</td>
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</table>

specifically include the serum samples that were necessary to demonstrate these problems, nor was the reproducibility of the various kits attempted in this study.

Several features of the kits may make them more or less attractive to routine diagnostic laboratories (Table 3). The three EIA kits required special equipment and were technically the most difficult, but they had the advantage of providing results within 2 to 2.5 h after the first incubation was begun. The Toxoplasma-G FIAAX test also required special equipment, but results were available within 1.5 h. The Cordia-T, Toxoelisa, and Toxoplasma-G FIAAX kits required mathematical manipulation of the raw data. Although computer software is available for the last two kits, additional expense is required for a small computer that has an interface with the reader. The LA and IHA test kits required the least amount of time and technical ability and no specialized equipment. However, because it was necessary to allow the LA test to incubate overnight, results were not available on the day of testing.

The Cordia-T, Toxoplasma-G FIAAX, Toxo Bio-EnzaBead, Toxoelisa, TMP-Test, and Eiken Toxotest-MT kits tested in this study detected the presence of Toxoplasma antibodies equally well. The sensitivity of the ToxHAtest could not be determined because both times the kit was used we were unable to read the cell patterns. For this study the results obtained by the IIF test, and not identification of Toxoplasma organisms, were used as the basis for determining the sensitivity and the specificity of the kits; therefore, the reactions detected by the Cordia-T and the Toxoelisa kits but not by the IIF test cannot be totally excluded as false positives.

Although manufacturers of all kits claimed that they were quantitative, the expression of quantitation differed among manufacturers, making comparison of results extremely difficult. A titer was obtained with the Toxoplasma-G FIAAX, Eiken Toxotest-MT, Toxo Bio-EnzaBead, and TPM-Test kits; but the Toxoelisa kit gave results in absorption units, and the Cordia-T test reported results in international units. Also, a titer obtained with a kit as compared with a titer obtained by IIF varied from manufacturer to manufacturer.

In determining the kit that is best for an individual laboratory, several factors must be considered: (i) type of population to be tested, (ii) whether a qualitative or quantitative test is necessary, (iii) the number of Toxoplasma tests to be performed, (iv) the technical skill available to perform the tests, (v) the equipment that is required, and (vi) the cost per test. About the same amount of time was required to perform tests with all kits; but the equipment needed, the technical degree of difficulty, and the cost per specimen varied greatly.

When it is important to determine how recently a Toxoplasma infection occurred, the results obtained with these kits are not adequate. Additional testing for Toxoplasma-specific IgM (5, 10) should be performed to provide more information on the time of infection.

TABLE 3. Comparison of commercial kits for detection of Toxoplasma antibody

<table>
<thead>
<tr>
<th>Comparison criteria</th>
<th>Cordia-T</th>
<th>Toxo Bio-EnzaBead</th>
<th>Toxoelisa</th>
<th>Eiken Toxotest-MT</th>
<th>Toxoplasma-G FIAAX</th>
<th>TPM-Test</th>
<th>ToxHAtest</th>
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<tbody>
<tr>
<td>Type of test</td>
<td>EIA</td>
<td>EIA</td>
<td>EIA</td>
<td>EIA</td>
<td>FIAAX</td>
<td>IHA</td>
<td>IHA</td>
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<td>Units of results</td>
<td>IU</td>
<td>Titer</td>
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<td>Titer</td>
<td>IHA titer</td>
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<tr>
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<tr>
<td>Test incubation time (h)</td>
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<td>2.17</td>
<td>2.25</td>
<td>12.00</td>
<td>1.50</td>
<td>2.00</td>
<td>1.00</td>
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<td>Technical degree of difficultya</td>
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<tr>
<td>Kit cost ($)b</td>
<td>394</td>
<td>115</td>
<td>167</td>
<td>100</td>
<td>120</td>
<td>123</td>
<td>61</td>
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<tr>
<td>Quantitative test cost ($)/specimen</td>
<td>3.83</td>
<td>5.00</td>
<td>1.94</td>
<td>1.82</td>
<td>2.40</td>
<td>6.14</td>
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</table>

a Subjective determination by the authors based on time and manipulations required: +, simple; + + + +, difficult.

b Cost as of April 1986.
LITERATURE CITED


