Serological Evaluation of Thin-Layer
Imunoassay–Enzyme-Linked Immunosorbent Assay
for Antibody Detection in Human Trichinellosis

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A new immunoenzymatic test, named the thin-layer immunoassay–enzyme-linked immunosorbent assay (TIA-ELISA), was evaluated for antibody detection in human trichinellosis using excretion and secretion products prepared from Trichinella spiralis muscle larvae. Serum samples from people with positive muscle biopsies or symptoms compatible with the disease (n = 8 or 26, respectively), all reactive in enzyme-linked immunoelectrotransfer blot assay (EITB), as well as 67 serum samples from healthy, EITB-negative people, were tested in an ELISA and TIA-ELISA. TIA-ELISA was performed in polystyrene plastic petri dishes by adding dots of 10 μl each of antigen (7 μg/ml) followed by adding diluted serum and the conjugate. Finally, the substrate mixed with agar was added to develop the reaction. Enzymatic by-products were easily detected by the naked eye as defined dots. Sensitivity and specificity were 76 and 94% for ELISA, and both parameters were 91% for TIA-ELISA. The kappa correlation indices for both tests in relation to EITB were 0.73 and 0.80, respectively. The TIA-ELISA can be carried out with common laboratory equipment in 3 h and uses lower quantities of antigen than EITB and ELISA. Since TIA-ELISA is easy to perform, cheap, sensitive, and specific, the test could be an acceptable alternative to use in clinical laboratories lacking specialized equipment needed for ELISA and EITB and in field studies for antibody detection in human trichinellosis.

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were collected, pooled, and clarified at 750 × g for 30 min. The reaction was stopped by adding 1 N NaOH. Absorbance values were obtained in an ELISA plate reader (Bio-Rad) at 405 nm. Samples were considered positive when absorbance values were higher than the cutoff value estimated as the mean plus 3 standard deviations of absorbance values from 15 negative control sera that were separately studied (4). Briefly, E/S antigen (3 μg/ml) was used to coat high-binding polystyrene plates (Costar, Cambridge, Mass.). After washing, serum samples were incubated for 120 min at 37°C.

Specific antibodies were detected using a goat anti-human immunoglobulin G- phosphatase conjugate (Sigma). Substrate solution containing p-nitrophenyl phosphate (Sigma) was used to develop the reaction by incubation at 37°C for 30 min. The reaction was stopped by adding 1 N NaOH. Absorbance values were obtained in an ELISA plate reader (Bio-Rad) at 405 nm. Samples were considered positive when absorbance values were higher than the cutoff value estimated as the mean plus 3 standard deviations of absorbance values from 15 negative control sera that were separately studied (x̄m = 0.183). TIA-ELISA. In order to determine the optimal conditions to perform the TIA-ELISA, several concentrations of the antigen and different solutions (PBS, 0.05% Tween in PBS, distilled water, 0.85% NaCl solution, and 0.1 M carbonate buffer) to coat the plastic surfaces of petri dishes were tested using pooled positive or negative sera. In addition, the optimal time and conditions for incubation of the reagents, the best dilution of serum samples and conjugate, and the usefulness of two chromogenic substances (p-phenylenediamine and 5-aminosalicylic acid) were also determined. The best conditions identified to perform the test were as follows. Sterile polystyrene petri dishes (Laboratorios Tecnica, Mexico City, Mexico) were firmly positioned on a pattern designed to accommodate 52 samples; the orientation was marked with a soft pen. Dishes were dotted on the marks indicated by the pattern with 10 μl of E/S antigen (7 μg/ml) diluted in 0.1 M carbonate-bicarbonate buffer (30 mM Na₂CO₃ and 70 mM NaHCO₃, pH 9.6) and incubated for 1 h at 37°C in a humidified atmosphere containing 95% air and 5% CO₂ for 48 h. Supernatants were collected, pooled, and clarified at 750 × g for 15 min. After protein precipitation with the Bradford dye reagent (Bio-Rad, Hercules, Calif.), a protein inhibitor cocktail (N-tosyl-l-phenylalanine-chloromethyl ketone and tosyl-l-lysine chloromethyl ketone, 50 μg of each per ml, and phenylmethylsulfonyl fluoride, 100 mM [final concentrations]) was added to the E/S antigen. The E/S antigens were kept frozen at −70°C in 1-ml aliquots until used.

ELISA. ELISA was performed as previously described (8). Briefly, E/S antigen (3 μg/ml) was used to coat high-binding polystyrene plates (Costar, Cambridge, Mass.). After washing, serum samples were incubated for 120 min at 37°C.

Specific antibodies were detected using a goat anti-human immunoglobulin G-phosphatase conjugate (Sigma). Substrate solution containing p-nitrophenyl phosphate (Sigma) was used to develop the reaction by incubation at 37°C for 30 min. The reaction was stopped by adding 1 N NaOH. Absorbance values were obtained in an ELISA plate reader (Bio-Rad) at 405 nm. Samples were considered positive when absorbance values were higher than the cutoff value estimated as the mean plus 3 standard deviations of absorbance values from 15 negative control sera that were separately studied (x̄m = 0.183).

**TABLE 1.** Serological evaluation of TIA-ELISA and ELISA in immunodiagnosis of human trichinellosis

<table>
<thead>
<tr>
<th>Test and result</th>
<th>Health condition of serum sample source</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
<th>Kappa coefficient index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected (EITB positive, n = 34)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIA-ELISA</td>
<td>Infected</td>
<td>91</td>
<td>91</td>
<td>84</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Normal (EITB negative, n = 67)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Positive</td>
<td>31</td>
<td>3</td>
<td>84</td>
<td>0.73</td>
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<tr>
<td></td>
<td>Negative</td>
<td>3</td>
<td>61</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Infected</td>
<td>76</td>
<td>94</td>
<td>87</td>
<td></td>
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<tr>
<td></td>
<td>Normal</td>
<td>26</td>
<td>4</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>8</td>
<td>63</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

Antigen-antibody reactions were visualized in TIA-ELISA as brown dots formed in the gel-containing substrate (Fig. 1). As is shown, the reaction zones are almost circular and are clearly differentiated from the neighboring clear areas. Color intensity, as well as the size and definition of the dots, is not uniform. Negative sera did not form colored spots. No changes in results were observed when complete dishes or just the gel-containing results were dried and preserved. Table 1 shows the reactivity of TIA-ELISA and ELISA compared to EITB, as well as the serological parameters analyzed. Sensitivity and specificity of TIA-ELISA were similar; in contrast, ELISA showed a lower sensitivity but a higher specificity. The predictive values both for positive and negative results are quite similar between the techniques, although for TIA-ELISA the negative predictive value is higher. The kappa coefficient indic-
ces obtained for both tests are considered good. Compared to the long time and high quantities of antigen and conjugate used by EITB (28 h and 2 μg of antigen and 1 μl of conjugate per sample) and ELISA (8 h and 0.63 μg of antigen and 0.1 μl of conjugate per sample), TIA-ELISA is a faster and more economic test, since it requires 3 h and 0.14 μg of antigen and 0.3 μl of conjugate per sample. Regarding the intensity of reaction by TIA-ELISA, 8 samples were classified as having a weak reaction, 10 had a medium reaction, and 19 had a strong reaction. However, no association was found among tests in relation to the intensity of the reaction (data not shown).

**DISCUSSION**

A novel immunoenzymatic test named TIA-ELISA was developed, standardized, and evaluated for antibody detection in human trichinellosis by using the E/S products of *T. spiralis* ML as antigens. Results were compared with those obtained with the conventional ELISA and were serologically evaluated using EITB as the reference test (9, 26). During the standardization process it was observed that the antigen could be easily attached to the plastic surface of the petri dish, especially when carbonate buffer was used. Also, the enzyme-substrate reaction took place in the dot area in the gel. Likewise, it was clearly determined that the peroxidase by-products remained stable in the dried gel, provided that 5-aminosalicylic acid was used as the chromogen as previously reported (4, 13, 15, 16).

An ideal serodiagnostic test, especially for developing countries, should be cheap, simple, and easy to perform, in addition to being sensitive and specific (28). As our results show, TIA-ELISA fulfills these requirements. The test could be as useful as EITB and ELISA for antibody detection in human trichinellosis, since the kappa coefficient index was higher than for ELISA and the sensitivity was high, meaning that TIA-ELISA can identify more positive samples than ELISA, which is relevant to selecting a screening test. Furthermore, TIA-ELISA is carried out using common laboratory equipment, and its results can be read by the naked eye in no more than 3 h, although this can be further reduced to almost 2 h if petri dishes are previously sensitized, because the antigenicity of the E/S antigens remains unaffected after drying. Moreover, the immune complexes and the peroxidase activity also remain stable after each drying step; in fact, Fig. 1 is a photograph of a dried gel in the petri dish from one experiment performed 10 months ago. Due to the above-mentioned reasons, TIA-ELISA may be a good candidate to be used in field studies, in which getting diagnostic information for cases of particular interest as fast as possible may be important in order to provide timely medical attention, as required in outbreaks, or to prevent the spread of human infections. Also, TIA-ELISA is a good alternative for clinical laboratories that lack equipment to perform EITB or ELISA. Finally, TIA-ELISA could also be useful for performing a faster diagnosis of *T. spiralis* infection in abattoirs before or immediately after slaughtering or in rurally reared or free-roaming swine to prevent transmission to humans (5, 6, 17, 24).

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**REFERENCES**