JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1991, p. 1831–1835
0095-1137/91/091831-05$02.00/0

Vol. 29, No. 9

# Immunodiagnosis of Toxocarosis in Humans: Evaluation of a New Enzyme-Linked Immunosorbent Assay Kit

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Received 15 January 1991/Accepted 11 June 1991

**Excretory/secretory (E/S) antigen derived from second-stage larvae of *Toxocara canis* maintained in defined medium in vitro has been well established worldwide for the immunodiagnosis of human toxocarosis by enzyme-linked immunosorbent assay. Such an enzyme-linked immunosorbent assay, based on the detection of human anti-*T. canis* (E/S antigen) serum immunoglobulin G, has recently been commercialized by Biokema-Affinity Products (Crissier-Lausanne, Switzerland). This commercial test kit was evaluated with regard to its application in a routine diagnostic laboratory and the reliability of the results. Of 78 patients with suspected clinical toxocarosis, 71 had anti-*T. canis* antibodies (positive serological result) corresponding to a diagnostic sensitivity of 91%; 14% of serum samples ($n = 199$) from patients with protozoan or with helminthic infections also showed positive reactions mainly related to infections with *Trichinella*, *Strongyloides*, and *Fasciola* species. An epidemiological study with 1,000 serum samples from randomly selected healthy blood donors and children in Switzerland demonstrated a seroprevalence of 2.7%. The test kit under evaluation had an overall diagnostic sensitivity of 91% and a relative specificity of 86%, the latter being related to some protozoan and helminthic infections. Because of the scarcity of such infections, potential cross-reactivity does not play a major role under the conditions found in the middle part of Europe. In conclusion, the application of the test kit provided for use in this study can be recommended for routine diagnostic use.**

Human visceral and ocular toxocarosis, which is caused by nematode larvae of the genus *Toxocara*, can occasionally be a serious or life-threatening condition (12, 23). *Toxocara canis*, an intestinal parasite of dogs, foxes, and other canids, has a worldwide distribution and is regarded as the main cause of human toxocarosis (1, 2, 7, 9). However, other species can be involved, such as *Toxocara mystax* from cats. In Switzerland, *Toxocara* infections in humans are relatively frequent, as indicated by a seroprevalence of 5% in blood donors (25). Excretory/secretory (E/S) antigen derived from second-stage larvae of *T. canis* maintained in defined medium in vitro (4, 5) has been used by various investigators for the specific immunodiagnosis of human toxocarosis by enzyme-linked immunosorbent assay (ELISA) (5, 11, 16, 22, 24). The immunochemical composition of these E/S antigens is well characterized (3, 18, 21). The defined technique for the production of *Toxocara* antigen and its excellent immunodiagnostic characteristics are suitable prerequisites for standardization and commercialization of the serological reagents. An ELISA based on the detection of anti-*T. canis* (E/S antigen) serum immunoglobulin G (IgG) has recently been commercialized by Biokema-Affinity Products (Crissier-Lausanne, Switzerland). The purpose of the present study was to evaluate this commercial test kit with regard to its application in a routine diagnostic laboratory and the reliability of the results that were obtained.

## MATERIALS AND METHODS

The nomenclature and taxonomy of parasitic diseases were based on the Standardized Nomenclature of Animal Parasitic Diseases rules (14), which are accepted by the World Association for the Advancement of Veterinary Parasitology and recommended by the World Federation of Parasitologists.

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**Sera. (i) Sera used for testing diagnostic sensitivity.** The sera selected from patients with suspected clinical toxocarosis were derived from three different studies performed previously. (i) Thirty serum samples originated from the study described by Speiser and Gottstein (24) and were obtained from patients with high antibody concentrations (ELISA) against *Toxocara* E/S antigen and who simultaneously had symptoms indicative of toxocarosis (eosinophilia, leukocytosis, respiratory signs, fever, hepatomegaly). (ii) Thirty pretreatment serum samples were selected from a study performed by Stürchler et al. (26) on the treatment of toxocarosis with albendazole. All patients showed symptoms or signs compatible with active toxocarosis as well as evident serological results in routine immunodiagnostic investigations. (iii) Sera from 18 symptomatic patients highly suspected of having toxocarosis were generously provided by M. Scaglia; these sera were derived from a recently described study (22). It must be mentioned that, currently, only a thorough clinical examination of the cases in combination with serology is the common way to confirm diagnostically cases of toxocarosis in humans, because the direct demonstration of the parasite in tissues of humans has been proven to be unlikely (5, 11).

**(ii) Sera used for testing specificity.** The sera used for the determination of cross-reactions were obtained from patients with clinically, parasitologically, or histologically proven infections with various helminths or protozoa. They were selected from the group of sera which was previously used for investigating the specificity of an ELISA by using *Echinococcus multilocularis* Em2 antigen (13) and *E. multilocularis* recombinant II/3-10 antigen (19). A detailed list of the parasite species involved is given in Table 2. The exclusion of a past or recent (asymptomatic) *Toxocara* infection was not possible.

**(iii) Sera used for the determination of normal ranges and parameters.** Sera from 500 healthy Swiss blood donors, matched by age (2 × 50 serum samples for each of the

TABLE 1. Results of *Toxocara* ELISA obtained with the sera from 78 patients with clinically suspected toxocarosis

| Sera of patients[a] | No.of serum samples | Diagnostic sensitivity (%) | $A_{405}$ | |
|---|---|---|---|---|
| | | | Mean | SD |
| Positive by ELISA | | | | |
| Positive | 28 | | 0.955 | 0.161 |
| Strongly positive | 43 | | 1.378 | 0.192 |
| Subtotal | 71 | 91 | | |
| Negative by ELISA | 7 | | 0.391 | 0.109 |
| Total | 78 | | | |

[a] Positive and negative were determined by following the manufacturer's criteria for interpretation.

following age groups: 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 to 69) and sex, were obtained from the Zürich Blood Donation Center. Sera from 500 children hospitalized for various reasons (data not shown) were obtained from the Children's Hospital of the University of Zürich (also matched in the same way as described above).

***Toxocara* ELISA.** An ELISA kit (catalog no. 9200, lot no. z119; Biokema-Affinity Products) for the detection of anti-*T. canis* (E/S antigen) serum IgG was used. Each kit provided the material to perform 96 individual ELISAs on polystyrene microtitration strips sensitized with *T. canis* E/S antigen. This antigen (ZH antigen) was basically generated by a previously described procedure (24) and was obtained from the same source described in that report (24). Sensitized strips were provided as eight-well modules in order to run assays for small series of samples. The presence of parasite-specific serum antibodies was detected with anti-human IgG-alkaline phosphatase conjugate.

The test procedure was performed as described by the manufacturer of the *Toxocara* ELISA kit. Briefly, the assay procedure was as follows: saturation with phosphate-buffered saline containing 0.3% Tween 20 and 0.1% bovine hemoglobin (incubation at room temperature) for 15 min; incubation (37°C) of the wells with 100 μl of the diluted sera (1/200) and the control sera, including a blank with no serum (phosphate-buffered saline containing 0.3% Tween 20 and 0.1% bovine hemoglobin) for 30 min; washing step; incubation (37°C) of 100 μl of diluted conjugate (conjugate was anti-human IgG-alkaline phosphatase) for 30 min; washing step; incubation (37°C) of 100 μl of substrate solution (1 mg of *p*-nitrophenylphosphate per ml of solution) for the chromogenic E/S reaction for 10 min; stop of the reaction by the addition of 100 μl of sodium hydroxyde solution; and reading of the $A_{405}$. The following modifications were made to this procedure. The high-positive reference control serum provided in the kit was calibrated to an $A_{405}$ of 1.0 instead of the value of 1.5 recommended by the manufacturer. This value corresponded to an incubation time of the substrate of 10 min.

For reading of the results for the test sera, the value of the no-serum blank had to be substracted from all measured values. Samples with an absorbance lower than that of the weak positive control serum had a parasite-specific antibody concentration that was considered to be nonsignificant for toxocarosis; interpretation was therefore negative. Samples with an absorbance higher than that of the weak positive control serum were regarded as positive; samples with absorbances higher than that of the positive control serum were regarded as strongly positive.

TABLE 2. Determination of the specificity of *Toxocara* ELISA[a]

| Infection group | No. of serum samples tested | ELISA results[b] | | | | % of positive reactions |
|---|---|---|---|---|---|---|
| | | No. negative | No. positive | | | |
| | | | 1 | 2 | Total | |
| Protozoan infections | | | | | | |
| Malaria | 10 | 10 | | | 0 | |
| Toxoplasmosis | 10 | 10 | | | 0 | |
| Amebiasis | 20 | 16 | 4 | 0 | 4 | |
| Subtotal | 40 | 36 | 4 | 0 | 4 | 10 |
| Trematode infections | | | | | | |
| Fascioliasis | 20 | 13 | 7 | 0 | 7 | |
| Schistosomiasis | 10 | 10 | | | 0 | |
| Subtotal | 30 | 23 | 7 | 0 | 7 | 23 |
| Cestode infections | | | | | | |
| Echinococcosis[c] | 50 | 50 | | | 0 | |
| Cysticercosis | 10 | 9 | | 1 | 1 | |
| Subtotal | 60 | 59 | 0 | 1 | 1 | 1.7 |
| Nematode infections | | | | | | |
| Ascariasis | 11 | 11 | | | 0 | |
| Strongyloidosis | 8 | 5 | 1 | 2 | 3 | |
| Trichinellosis | 40 | 28 | 4 | 8 | 12 | |
| Filariasis | 10 | 9 | 1 | | 1 | |
| Subtotal | 69 | 53 | 6 | 10 | 16 | 23 |
| Total | 199 | 171 | 17 | 11 | 28 | 14 |

[a] The total relative specificity was 86%.

[b] Positive and negative were determined by following the manufacturer's criteria for interpretation: 1, positive reaction; 2, strongly positive reaction.

[c] The *Echinococcus* infections were as follows: *E. granulosus* ($n$ = 30) and *E. multilocularis* ($n$ = 20).

In addition to the control sera provided in the test kit, we included the following laboratory controls in each test run: one negative reference serum, one highly positive serum sample from a patient, and eight titrated solutions of the positive serum sample from a patient diluted with the negative reference serum (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256). $A_{405}$ values were read by using a Dynatech MR.5000 reader with the software version 2.6.

## RESULTS

**Diagnostic parameters.** The overall diagnostic sensitivity obtained with the test kit used in this study was 91%, and the

TABLE 3. Results of *Toxocara* ELISA obtained with sera from 500 healthy Swiss blood donors and 500 Swiss hospitalized children

| Serum group[a] | No. of serum samples tested | % Seroprevalence | $A_{405}$ | | Avg age (yr) |
|---|---|---|---|---|---|
| | | | Mean | SD | |
| Blood donors | | | | | |
| Positive by ELISA | 20 | 4.0 | 0.852 | 0.250 | 49 |
| Negative by ELISA | 480 | | 0.066 | 0.083 | 44 |
| Children | | | | | |
| Positive by ELISA | 7 | 1.4 | 0.914 | 0.130 | 9.7 |
| Negative by ELISA | 493 | | 0.082 | 0.096 | 7.5 |
| Overall population | 1,000 | 2.7 | | | |

[a] Positive and negative were determined by following sthe manufacturer's criteria for interpretation.

TABLE 4. Relation between the absorbance obtained for populations with negative ELISA results and the cutoff value of a diagnostic test based on the weak positive control serum

| Serum group | $A_{405}$ | | | |
|---|---|---|---|---|
| | Mean | SD | Mean + 3 SD | Effective SD related to the absorbance of the discriminating weak positive control serum |
| Negative ELISA | | | | |
| Children | 0.082 | 0.096 | 0.370 | 4.7 |
| Adults | 0.066 | 0.083 | 0.315 | 5.6 |
| Control provided with kit[a] | 0.532 | | | |

[a] The control serum was declared weakly positive by the manufacturer and served to provide a cutoff value.

relative specificity was 86%; the latter was with regard to potential cross-reactions caused by other parasitic infections (Tables 1 and 2). These potential cross-reactions mainly occurred in patients with *Trichinella spiralis* and trematode infections. However, a simultaneous toxocarosis could not be excluded in these individuals; therefore, the specificity value was relative.

The overall seroprevalence was 4.0% in the adult Swiss population and 1.4% in children hospitalized for various reasons (Table 3). The absorbance data obtained from this seroprevalence study demonstrated that the absorbance of the weak positive control serum (cutoff) was higher than the mean value plus 3 standard deviations of the absorbance observed in a negative population (Table 4). Note the correspondence between the mean value and the standard deviation of the absorbance obtained for a negative population and the absorbance of the cutoff value that was obtained. Therefore, the overall seroprevalence of anti-*T. canis* (E/S antigen) serum IgG of this Swiss population was 2.7%. No significant differences in test prevalences were observed with respect to gender or age distribution in the group of adults.

On the basis of a seroprevalence of 2.7% obtained by the ELISA in the Swiss population, we determined the predictive value of a negative result to be 99.7%. Because of the relativity of the value of specificity (86%), which was based on a group of patients selected for potential cross-reactions and, thus, did not reflect the prevalences of the respective diseases, we did not calculate the predictive value of a positive result.

TABLE 5. Quality control intertest variation determined by the investigating control sera (kit standards and IcS) assayed in 19 ELISA runs

| Serum group | $A_{405}$ | | | | CV (%) |
|---|---|---|---|---|---|
| | Mean | SD | Minimum | Maximum | |
| Control provided with kit | | | | | |
| Negative | 0.112 | 0.022 | 0.077 | 0.167 | 20 |
| Positive | 0.532 | 0.075 | 0.446 | 0.695 | 14 |
| Strongly positive[a] | 1.012 | 0.114 | 0.754 | 1.212 | 11 |
| Laboratory standards | | | | | |
| Negative | 0.033 | 0.043 | 0.001 | 0.173 | —[b] |
| IcS1 | 1.475 | 0.115 | 1.262 | 1.648 | 8 |
| IcS2 | 1.323 | 0.126 | 1.153 | 1.549 | 10 |
| IcS4 | 1.070 | 0.100 | 0.932 | 1.228 | 9 |
| IcS8 | 0.833 | 0.086 | 0.708 | 0.997 | 10 |
| IcS16 | 0.567 | 0.078 | 0.466 | 0.734 | 14 |

[a] Positive and strongly positive were determined by following the manufacturer's criteria for interpretation.

[b] —, not calculated, because of the low level of the absorbance.

TABLE 6. Intratest variation determined with control sera (IcS) assayed in one ELISA run and in one plate for 20 groups of four serum samples each

| Serum group | $A_{405}$ | | | | CV (%) |
|---|---|---|---|---|---|
| | Mean | SD | Minimum | Maximum | |
| IcS3 | 1.190 | 0.036 | 1.131 | 1.264 | 3.0 |
| IcS5 | 0.625 | 0.018 | 0.602 | 0.668 | 2.9 |
| IcS7 | 0.269 | 0.019 | 0.244 | 0.308 | 7.0 |
| IcS9 | 0.082 | 0.012 | 0.070 | 0.107 | 14.2 |

**Laboratory parameters.** For analyzing inter- and intratest variations, we introduced some internal control sera (IcS), which were described in Materials and Methods. The results in Table 5, which were obtained from 19 different plates (corresponding to individual ELISA runs), show the intertest variations, whereas intratest variations are given in Table 6.

The coefficients of variation (CV) ranged between 8 and 20% for the intertest variation and between 3 and 14% for the intratest variation. The CV depended on antibody concentrations in relation to absorbance values, with the CV increasing with respect to a decrease in $A_{405}$ values.

## DISCUSSION

Under the conditions found in Europe, the cross-reactivities with sera from patients with strongyloidosis, trichinellosis, filariasis, and fascioliasis do not play a significant role in routine diagnosis, because autochthonous infections are extremely rare or event absent. The situation is different in areas with high prevalences of these infections, especially with regard to strongyloidosis and filariasis. In all cases in which potential cross-reactivity has been detected, an unidentified *Toxocara* infection could not be excluded with certainty (15).

Our values of diagnostic sensitivity and specificity are in accordance with those obtained in other studies with the same type of antigen (Table 7). Glickman et al. (11), however, reported a lower diagnostic sensitivity and a higher specificity. In general, few hard data on the reliable demonstration and characterization of the operating characteristics of *Toxocara* ELISA with the E/S antigen are available. This may be related to the known difficulties of sampling well-documented groups of patients with toxocarosis as well as to the difficulty of excluding a past *Toxocara* infection in

TABLE 7. Results (diagnostic sensitivity and specificity) obtained by various investigators for the immunodiagnosis of toxocarosis

| Reference | Test (antigen) | Percent | |
|---|---|---|---|
| | | Sensitivity | Specificity |
| Glickman et al. (11) | ELISA (E/S antigen) | 78.3 | 92.3 |
| | Ouchterlony (E/S antigen) | 65.2 | 94.9 |
| Speiser and Gottstein (24) | ELISA (E/S antigen) | 80 | 93 |

TABLE 8. Comparison of some arbitrarily selected seroprevalences from different countries[a]

| Country | % (no.) of the following population: | | Reference |
|---|---|---|---|
| | Children | Adult | |
| Australia | | 7 (660) | 20 |
| Great Britain | | 2.6 (922) | 5 |
| Italy | | 4.2 (324) | 6 |
| Japan | 3.6 (83) | 4 (530) | 17 |
| Sudan | | 5 (962) | 28 |
| Switzerland | | 5 (765) | 25 |
| The Netherlands | 7.1 (112) | | 27 |
| United States | 8 (100) | | 8 |
| | | 2.8 (8,590) | 10 |

[a] *Toxocara* (E/S) antigen and ELISA were used in these studies.

patients whose sera are used for investigating cross-reactivity. Results of the present investigation basically appear to be in agreement (with respect to diagnostic sensitivity and specificity) with those from a previous study (24). This can be explained by the use of identical crucial reagents (ZH antigen and alkaline phosphatase-conjugated anti-human IgG second antibody) in both test systems. At this point, it should be mentioned that one of the major objectives for commercialization of a test kit for the diagnosis of human toxocarosis concerns the availability of standardized E/S antigen, which is offent difficult and time-consuming to make and may be impossible for many laboratories to generate. Concerning the manufacturer's definition of positive and negative test results, Table 3 explains the relation between a theoretical cutoff (mean plus 3 standard deviations, which is commonly used) and the cutoff point defined by the manufacturer (corresponding to the weak positive control serum). It shows that the test cutoff point includes a large security zone (approximately 5 standard deviations).

The results concerning the seroprevalences in blood donors correspond approximately to those obtained by other investigators in European countries as well as in the United States. Surveys of adults in the United States indicated that 2.8% had significantly elevated levels of anti-*T. canis* antibodies (10), while reports from Australia (20) and The Netherlands (27) indicated rates of about 7% (Table 8).

Globally, the results depicted in the present study are in agreement with those published by various investigators who used the same type of E/S antigen as that used in the test kit described here. In conclusion, the application of the test kit provided for use in this study can be recommended for the routine diagnosis of *Toxocara* infections in humans.

## ACKNOWLEDGMENTS

We are grateful to M. Scaglia (Pavia, Italy), N. Nadal (Kinderspital Zürich), and R. Schenker (Blutspendezentrum Zürich) for providing sera and to C. Bordier (Biokema-Affinity Products) for providing free diagnostic kits.

We thank Judith Lauffer and Mirka Schmid for competent technical assistance.

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