ELISA for toxoplasma antibody detection: a comparison with other serodiagnostic tests

A BALSARI,* G POLI,* V MOLINA,† M DOVIS,‡ E PETRUZZELLI,‡
A BONIOLO,§ AND E ROLLERI§

From the *Istituto di Microbiologia e Immunologia Veterinaria dell'Università, Via Celoria 10, Milan;
†Centro Analisi Mediche, Piazza Trente e Trieste, Monza; ‡SORIN Biomedica, Saluggia (VC);
§Clinical Immunochemistry Unit SORIN Biomedica—Laboratorio Fisiologia Clinica CNR, Saluggia and
Pisa, Italy

SUMMARY An ELISA method was developed for the measurement of toxoplasma IgG antibodies
in human serum using antigen-coated polystyrene beads as a solid phase and anti human IgG-horse
radish peroxidase conjugate as an enzymatic tracer. In order to assess ELISA sensitivity and
specificity, a between methods comparison was made using 'conventional' serological tests as
reference (dye-test, crossover-linked immunoassay, passive haemagglutination, indirect immuno-
fluorescence). From an analysis of the group classifications obtained some considerations emerged:
the ELISA specificity looks comparable with that of the 'reference' tests, as no sample classified as
negative by all these tests was ELISA-positive, and vice versa; ELISA appears to correlate better
with haemagglutination and immunofluorescence, on the basis of the respective class frequencies;
in particular, the number of positives, which is much lower for the dye-test and crossover-linked
immunoassay, suggests that a higher sensitivity is reached in the former cases.

None of the several tests mostly used for the sero-
diagnosis of toxoplasmosis appears fully adequate
for mass-screening purposes as far as analytical
reliability, experimental ease, and promptness of
response are concerned. As a matter of fact some of
them need special equipment not widely available,
and others cannot be used as single tests owing to
the incompleteness of the information obtainable
and the poor correlation with the clinical situation.

Recently, the enzyme-linked immunosorbent assay
(ELISA) has been proposed as a promising sero-
logical test for infective and infestive diseases.1–5
Also in our laboratories attempts were made to
evaluate the actual potential of ELISA in the
diagnosis of toxoplasma infections. In particular,
this study was aimed at defining the specificity and
sensitivity of an ELISA method we have recently
developed through a comparison with other sero-
logical tests assumed as references, such as the dye-
test, crossover-linked immunoassay, indirect haem-
agglutination, and indirect immunofluorescence.

Material and methods

SAMPLES
The samples were taken at random among human
sera sent for toxoplasma serodiagnosis to diagnostic
centres in Milan.

ELISA REAGENTS AND PROCEDURE

Antigen
Soluble extracts from trophozoites of Toxoplasma
gondii, RH strain, harvested from the peritoneal
exudate of mice 72 hours after injection, were
prepared by sonication disruption of cells, followed
by centrifugation. A solution of 10 μg protein/ml
in Tris-HCl buffer, pH 7.2, was used for coating.

Solid phase
Polystyrene beads, ca 6 mm diameter (Precision
Plastic Balls, USA), were sensitised through over-
night immersion in the coating solution at room
temperature, followed by washing with deionised
water and vacuum-drying.

Received for publication 6 December 1979
Enzyme-labelled anti human globulins
Horse radish peroxidase (HRP, Boehringer, FRG) and rabbit anti human IgG, purified by affinity chromatography, were coupled using the periodate method slightly modified to obtain an approximate HRP/IgG molar ratio of 2. The conjugate was purified through gel-filtration on Sephadex G-200 (Pharmacia, Sweden) and used in the assay at a dilution of approximately 0·4 µg/ml in 0·05 M phosphate buffer, pH 7·4, containing 4% bovine serum albumin (BSA) and 0·01% merthiolate.

Substrate for the enzymatic reaction
150 mg of O-phenylene-diamine dihydrochloride (home-prepared from the free base) were added before use to 50 ml of 0·25 M phosphate-citrate buffer containing 0·02% H2O2, to obtain a final pH of 4·8.

Diluent system for serum samples
0·05 M phosphate buffered saline, pH 7·4, 4% BSA, 0·01 M EDTA, 0·1% NaN3, was used.

ELISA procedure
The following scheme was adopted:
—1st incubation: test solution 15 min, 40°C (0·2 ml 1:10 diluted sample for each bead)
—Washing with deionised water 4 x 2·5 ml
—2nd incubation with enzymatic tracer (0·2 ml)
—Washing with deionised water 4 x 2·5 ml
—3rd incubation with substrate 10 min, room temperature (0·5 ml)
—Stop with 2 N H2SO4 (1·0 ml)
—Absorbance readings at 492 nm

The ELISA data were expressed as ELISA mU, ie, absorbance x 106.

‘REFERENCE’ SEROLOGICAL TESTS
The procedure reported by Sabin and Feldman was followed for the dye-test (DT). The passive haemagglutination test (HA) was performed using the Toxo-HA kit supplied by BioMerieux (France). For the indirect immunofluorescence (IF) test the IF test of Roche Diagnostics (USA) was used. The crossover-linked immunoassay (cLIA) was carried out according to the procedure described by Zotti et al. using the same antigen preparation as in the case of ELISA.

Results and discussion
Eighty-six serum samples were tested with the reference methods and accordingly classified as three distinct groups: negative, ‘borderline’ (ie, weakly positive), and positive. The classification criteria for each method and the resulting distribution of samples are reported in Tables 1 and 2, respectively. The latter table shows that 16 samples are classified as negative and 12 as positive by all the reference methods (‘reliably’ negative and ‘reliably’ positive).

Table 1 Classification criteria of the ‘reference’ tests

<table>
<thead>
<tr>
<th>Sample classification</th>
<th>Test response (reciprocal titres)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DT</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Borderline</td>
<td>50-100</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

*As in this case the sera are tested undiluted, 1/4 diluted, and 1/16 diluted, the results are expressed as the maximal dilution still corresponding to a visible precipitation.

Table 2 Classification of the samples (n = 86) according to the ‘reference’ tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>DT</td>
<td>39</td>
</tr>
<tr>
<td>cLIA</td>
<td>47</td>
</tr>
<tr>
<td>HA</td>
<td>24</td>
</tr>
<tr>
<td>HA + cLIA</td>
<td>35</td>
</tr>
<tr>
<td>IF</td>
<td>24</td>
</tr>
<tr>
<td>HA + IF</td>
<td>17</td>
</tr>
<tr>
<td>ALL</td>
<td>16</td>
</tr>
</tbody>
</table>

The 16 reliably negative samples gave in the ELISA system a mean value and an SD of 126 ± 17·5 mU. Therefore, assuming a 1% probability of misclassification (ie, x ± 4 SD), the upper limit for the negative population is 196 mU. For the 12 reliably positive samples mean and SD were 469 ± 124 mU. This corresponds to a distribution ranging, at a 95% probability (x ± 2 SD to x + 2 SD), from 221 to 717 mU without any overlap with the negative class.

From an analysis of the ELISA data, imposing the 196 mU limit for the negative population, 25 out of 86 samples were negative. A comparison of these results with those given by the other methods (Table 3) demonstrated that (a) no ELISA negative sample was classified as positive by any other method, and (b) nine negatives were seen as borderline by one or two reference methods (ie, 5 IF, 2HA, and 2 cLIA borders).

Taking into account all the reference tests combined together, no ‘reliably’ borderline population can be identified (Table 2). Therefore, to complete
Table 3  Analysis of the ELISA negatives (n = 25) according to the 'reference' tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency</th>
<th>Negative</th>
<th>Borderline</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>cLIA</td>
<td>23</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>23</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>20</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

In the evaluation, the tests were grouped according to their stated levels of sensitivity. In this way 20 borderline samples resulted, combining HA and IF tests. This population gave mean and SD ELISA values of 299 ± 49 mU, corresponding to a distribution ranging, at a 95% probability, from 201 to 397 mU. It is apparent that this population is completely discriminated from the negative one, while some overlap exists with the positive class. A range for the borderline samples could be tentatively assumed to be 200-350 mU (ie, from the negative-borderline cut-off to \( x + SD \) where \( x \) refers to the mean ELISA response of HA-IF borderlines).

By applying the same analysis to the 16 samples classified as borderline by DT and cLIA, a mean value of 374 ± 77 mU and a 95% probability range 220-528 were obtained. Also in this case no overlap with the negative population can be observed, but borderlines and positives largely superimpose.

The classification of the 86 samples according to the ELISA response is shown in Table 4 as resulting from the above data, while the Figure illustrates the correlations obtained.

Some conclusive considerations can be attempted. In terms of specificity, ELISA looks comparable with the other tests (if not superior) as no sample classified

![Graphs showing ELISA responses with reference tests](http://jcp.bmj.com/)

Correlation of the ELISA responses with the results of the reference tests. The class subdivision on each scale (negative, borderline, positive) is indicated by dotted lines.

*The doubtful precipitation arcs, which are indicated as + for undiluted and * for 1/4 diluted serum, were ascribed to negative and borderline classes, respectively. + represents a positivity with the diluted serum.
ELISA for toxoplasma antibody detection: a comparison with other serodiagnostic tests

Table 4  Classification of the samples (n = 86) according to the ELISA response (mU)

<table>
<thead>
<tr>
<th>Sample classification</th>
<th>Frequency</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>25</td>
<td>126 ± 18</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Borderline</td>
<td>34</td>
<td>307 ± 24</td>
<td>200-350</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>427 ± 45</td>
<td>&gt;350</td>
</tr>
</tbody>
</table>

as negative by all these tests was ELISA-positive, and vice versa; ELISA appears to correlate better with HA and IF tests than with DT and cLIA, as indicated by the frequency in the respective group classifications; in particular, the number of negatives, much lower for ELISA, IF, and HA than for DT and cLIA, suggests that higher sensitivity levels are obtained in the former cases.

Conclusions

The results of the present study indicate a substantial agreement in group classification of samples between the ELISA developed in our laboratories and conventional serological tests for toxoplasmosis. This holds particularly in respect of indirect immunofluorescence and passive haemagglutination, which are to be regarded as suitable tests.9 10

The ELISA conditions adopted were directed to obtain a high practicability level rather than to exploit the full potential of the method. Work is presently in progress to optimise further the ELISA performances as for sensitivity (incubation time, sample dilution), response precision, and consistency (reagent stability, way of expressing data), and, especially, possibility of discriminating between IgG and IgM antibody classes.

This work was supported in part by the Special Project on Biomedical Engineering, National Research Council—CNR, sub-project Chim. 2.

References


Requests for reprints to: Professor G Poli, Università di Milano, Facoltà di Medicina Veterinaria, Istituto di Microbiologia e Immunologia, Via Celoria 10, Milan, Italy.
ELISA for toxoplasma antibody detection: a comparison with other serodiagnostic tests.
A Balsari, G Poli, V Molina, M Dovis, E Petruzzelli, A Boniolo and E Rolleri

doi: 10.1136/jcp.33.7.640

Updated information and services can be found at:
http://jcp.bmj.com/content/33/7/640

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/