A comparison of isotopic and enzyme-immunoassays for tropical parasitic diseases

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Summary

A comparison is made of enzyme-immunoassay and radio-immunoassay for the detection of antibody in Chagas's disease, sleeping sickness, malaria, schistosomiasis and invasive amoebiasis. Both assays were sensitive and reproducible and gave comparable results.

Introduction

In temperate climates isotopic assays are employed routinely for the diagnosis of many diseases but they have been little used for the detection of those diseases caused by parasites in the tropics. This is partly due to the high cost and short shelf life of reagents used in the isotopic assays and it has to be accepted that there has been little economic incentive for commercial organizations to produce test-kits for radio-immunoassays for parasitic infections.

This paper deals with both isotopic and enzyme immunoassays for detecting antibody in parasitic infections and a critical comparison is made of the results obtained with each.

Materials and methods

For both the isotopic and enzyme-linked immunoassay methods (ELISA) the indirect microplate method (VOLLER et al., 1976a) was used as follows:

(1) 200 µl of antigen (diluted in coating buffer) was incubated in each well of disposable polystyrene micro- haemagglutination plates (Microtiter M220-29, Dynatech Labs) overnight at +4°C.

(2) The plates were then washed in PBS Tween (phosphate buffered saline pH 7.4, 0.015M, 0.05 % Tween 20, 0.02 % NaNO₃). This was achieved by emptying the plate contents then refilling the wells with PBS-Tween, leaving for three minutes and again emptying. This was repeated three times. Finally the plates were shaken dry and the next reagent (see 3) was added.

(3) 200 µl of serum or plasma diluted in PBS-Tween was added and incubated for two hours at 37°C.

(4) The washing step (2) was repeated.

Subsequent steps differed depending on whether an isotopic assay of ELISA was carried out.

Isotopic assay

(5a) 200 µl of a diluted preparation of ¹²⁵I labelled sheep antihuman IgG was added to each well. The plate was then incubated at 37°C for two hours.

(6a) The plate was then washed as before and dried. Each well was then filled with paraffin wax and the wells were separated (by cutting the plate with scissors). Each well was placed in an outer plastic tube and the radioactivity was assessed by counting for two minutes in a gamma counter (WALLAC-DECEM GTL). The results were expressed as counts per minute (cpm).

ELISA

(5b) 200 µl of a diluted conjugate of alkaline phosphatase labelled sheep antihuman IgG was added to each well and the plate was then incubated for two hours at 37°C.

(6b) The plate was then washed as before and shaken dry.

(7b) 200 µl of the enzyme substrate was added to each well and was incubated for 30 minutes at room temperature. The enzyme substrate was p-nitrophenyl phosphate (Sigma 104 phosphatase substrate tablets were used. One 5 mg tablet was dissolved just before use in 5 ml of 10% diethanolamine buffer (97 ml diethanolamine, 800 ml H₂O, 0.2g NaN₃, 100 mg Cl₂6H₂O, 1M HCl added to give pH 9.8 and made up to one litre with H₂O; stored at +4°C in the dark).

(8b) 50 µl of 3M NaOH was added to each well to stop the reaction. The contents of each well were then transferred to a microcell in a spectrophotometer (VITATRON UPM) and the absorbance was read at 405 nm. Samples giving readings over 1.0 were diluted with distilled water until readings were under 1.0 then the original absorbance was calculated. The results were expressed as O.D. or E/405 units.

Labelled Reagents

(1) Enzyme conjugate

The IgG fraction of a sheep antiserum to human immunoglobulin was labelled, for ELISA tests, with alkaline phosphatase by the one-step gluteraldehyde
method of AVRAMEUS (1969). The enzyme-antibody conjugates were standardized according to the guidelines given by VOLLE et al. (1976b) and were diluted appropriately in PBS-Tween. The working dilution for the conjugate used in these tests was 1/2000. The isotope labelled reagent (see below) was diluted to give approximately the same molar concentration of antibody.

(2) $\text{I}^{125}\text{ labelled immunoglobulin}$

The IgG fraction of the same sheep antiserum to human immunoglobulin was precipitated with 18% Na$_2$SO$_4$ and was labelled with $\text{I}^{125}$ by the method of KARONE et al. (1975). The $\text{I}^{125}$ labelled IgG was purified on Sephadex G100 before use in the assay. For use the labelled preparation was diluted to give the same molar concentration (with respect to IgG) as that of the enzyme conjugate.

(3) $\text{I}^{125}$ labelled sheep antibody (purified and specific to human immunoglobulin)

Specific antibody to human immunoglobulin was isolated on an immunoadsorbent (Cellulose-human-IgG). It was iodinated with $\text{I}^{125}$ whilst bound to the solid phase and it was recovered by dissociating the complex at low pH by the method of O’RIORDAN & WOODHEAD (1975).

Antigens

The following antigens were used.
(1) Trypanosoma cruzi from culture for detecting antibody in Chagas’s disease.
(2) Trypanosoma brucei from infected mice for detecting antibody in sleeping sickness.
(3) Plasmodium falciparum from blood of infected Aotus for detecting malarial antibody.
(4) Schistosoma mansoni soluble egg antigen. Kindly donated by Dr. Draper for the schistosomiasis tests.
(5) Entamoeba histolytica prepared from axenic cultures for the serology of invasive amoebiasis.

The detailed preparation of each antigen is described by VOLLE et al. (1976a).

Sera

Sera from the following groups were used. All were from adults.
(1) Brazilians with Chagas’s disease.
(2) Brazilians without Chagas’s disease.
(3) East Africans with T. rhodesiensc sleeping sickness.
(4) East Africans without sleeping sickness.
(5) New Guinea lowlanders from an area of high transmission of P. vivax and P. falciparum.
(6) New Guinea highlanders from a non-malarious area.
(7) Europeans with amoebic liver abscess.
(8) Healthy adult Europeans resident in Britain.

Results

(a) General

The results of the isotopic assay and ELISA on T. cruzi antigen with several dilutions of sera, from three people with Chagas’s disease and one normal individual, are shown in Fig. 1. The isotopic assay and ELISA gave similar results. At dilutions of 1/50,000 to 1/100,000 the positive sera had values comparable to those of the control serum only diluted 1/200. Similar titrations were carried out in all the other antibody/antigen systems.

Fig. 1. Results of isotopic and enzyme immunoassays for Chagas’s disease.
investigated in this study and comparable results were obtained. The close correlation between the $^{125}$I labelled globulin and the ELISA results is emphasized by the plotting one against the other (Fig. 2). The correlation coefficient in the $T. cruzi$ system illustrated was 0.997. Correlation coefficients between ELISA and $^{125}$I assays of over 0.98 were obtained for the tests for antibody to malaria parasites, trypanosome, schistosomes and amoebae.

The very close correspondence of results obtained by using isotope labelled whole immunoglobulin and $^{125}$I labelled purified antibody are shown in Fig. 3. The illustration is of tests carried out on $E. histolytica$ antigen but similar results were obtained in all the other systems examined. Because of this all subsequent tests were carried out with the labelled immunoglobulin fraction which is easier and cheaper to prepare than specific antibody.

(b) Chagas’s disease

Both ELISA and $^{125}$I assays were carried out with $T. cruzi$ antigen on sera from a group of Brazilians thought to have Chagas’s disease (because of their clinical condition and because they were positive by IFA, IHA and CFT). They were compared with a group of Brazilians known to be free from Chagas’s disease. The results in Fig. 4 show that all the Chagas’s disease patients had higher values than the controls. There were no cross-reactions with sera from patients with malaria but, as expected, the sera of patients with African sleeping sickness gave positive results (Table 1).

(c) African Trypanosomiasis

The ELISA and isotopic assay values, on $T. brucei$ antigen, are given for a group of patients with sleeping sickness and European and African controls (Fig. 5). The cross-reactivity of the $T. brucei$ antigen was investi-
COMPARISON OF ISOTOPIC AND ENZYME-IMMUNOASSAYS FOR TROPICAL PARASITIC DISEASES

Table I – Results of ELISA and isotopic assays with T. cruzi antigen

<table>
<thead>
<tr>
<th>Number</th>
<th>Isotopic assay (c/p/m)</th>
<th>ELISA (E/405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European controls 3</td>
<td>120±18</td>
<td>0·151±0·016</td>
</tr>
<tr>
<td>Brazilian controls 16</td>
<td>247±11</td>
<td>0·248±0·09</td>
</tr>
<tr>
<td>Malaria cases 3</td>
<td>234±53</td>
<td>0·278±0·09</td>
</tr>
<tr>
<td>Sleeping sickness cases 3</td>
<td>868±258</td>
<td>1·47±0·86</td>
</tr>
<tr>
<td>Brazilians with Chagas’s disease 19</td>
<td>2,091±245</td>
<td>4·08±0·80</td>
</tr>
</tbody>
</table>

Table II – Results of ELISA and isotopic assays with T. brucei antigen

<table>
<thead>
<tr>
<th>Number</th>
<th>Isotopic assay (c/p/m)</th>
<th>ELISA (E/405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European controls 3</td>
<td>154±23</td>
<td>0·10±0·034</td>
</tr>
<tr>
<td>African controls 8</td>
<td>424±33</td>
<td>0·42±0·140</td>
</tr>
<tr>
<td>Malaria cases 3</td>
<td>325±15</td>
<td>0·28±0·12</td>
</tr>
<tr>
<td>Patients with schistosomiasis 6</td>
<td>311±88</td>
<td>0·29±0·17</td>
</tr>
<tr>
<td>Brazilians with Chagas’s disease 3</td>
<td>1,385±643</td>
<td>1·26±0·78</td>
</tr>
<tr>
<td>Sleeping sickness cases 23</td>
<td>1,491±401</td>
<td>1·32±0·36</td>
</tr>
</tbody>
</table>

Fig. 3. Correlation of isotopic assays for amoebiasis with 1125 labelled immunoglobulin and with 1125 labelled specific antibody conjugates.

Fig. 4. A comparison of isotopic and enzyme immunoassays for Chagas’s disease.

Fig. 5. A comparison of isotopic and enzyme immunoassays for African trypanosomiasis.
(d) **Malaria**

The distribution of ELISA and isotopic assay values obtained with *P. falciparum* antigen tested against sera from New Guinea highlanders living in a non-malarious area, and from New Guinea lowlanders from a malaria endemic area are shown in Fig. 6. The latter had much higher values in both assays.

There were no cross-reactions with sera from patients with schistosomiasis or with Chagas’s disease (Table III).

(e) **Schistosomiasis**

For the tests with *S. mansoni* antigen a preparation of isotope labelled globulin with higher activity was used. There was again a close correlation between ELISA and isotopic assays. The sera from the patients with proven schistosomiasis gave much higher values in both tests (Fig. 7) and there were no cross-reactions with malaria or Chagas’s disease patients sera. The small group of sleeping sickness patients had higher values than normal individuals in both assays (Table IV).

(f) **Invasive amoebiasis**

Both ELISA and the $^{125}$I assays were equally effective in detecting the patients with invasive amoebiasis when
E. histolytica was used (Fig. 8). There were no cross-reactions with malaria, Chagas’s disease or schistosomiasis but again, sleeping sickness patients sera gave higher values (Table V).

The present study shows that these conjugates give identical results to those obtained with the isotopically labelled antibody preparations which have been found over many years’ experience to give reliable results. Furthermore our present study shows that in the context of detecting antibody in infectious diseases it is not necessary to purify the antibody for the enzyme conjugation. The labelled immunoglobulin fraction was quite as satisfactory as the labelled purified antibody and the former was much easier to prepare. However, it may be necessary to utilize purified antibody in those situations where much greater sensitivity (e.g. hormone assays) is required.

We have shown that radio-immunoassay is perfectly satisfactory for the measurement of antibody in parasitic diseases but there is no doubt that ELISA has several inherent advantages over RIA. ELISA is simple and safe and the reagents are cheap and have a long shelf life. The fact that for many purposes ELISA results can be read by eye also commends it rather than RIA for use in developing countries.

The test for antibody in American and African trypanosomiasis confirmed our earlier findings (VOLLER et al., 1975b; VOLLER et al., 1976a) that extracts of the culture forms of T. cruzi and of blood forms of T. brucei yield highly reactive soluble antigens. Both isotopic and enzyme immunoassays revealed cross-reactions but this is not unexpected since T. cruzi and African trypanosomes are phylogenetically related. In practice their geographical separation means that these cross-reactions would not pose diagnostic problems. WHO (1976a) recently evaluated all the serological tests at present used for sleeping sickness and it was found that ELISA was the most sensitive method for detecting antibody in this disease. LUCKINS (1977) has since shown that ELISA is also sensitive in detecting infected cattle.

Malarial antibody was equally well detected, using P. falciparum antigen, with ELISA and with the isotopic assay and individuals from malarious areas could be clearly distinguished from uninfected controls. Similarly ELISA and isotopic assays for amoebic antibody with E. histolytica antigens were equally satisfactory in indicating invasive amoebiasis. Bos et al. (1975) also reported good results with ELISA in detecting antibodies to E. histolytica.

Our results confirm those of SCHINSKI et al. (1976) who found that both ELISA and RIA were sensitive and reliable in detecting antibody to schistosomiasis. We agree with SCHINSKI and colleagues (1976), with HULD T et al. (1975) and MCLAREN et al. (1977) in the view that ELISA may be particularly suitable for epidemiological surveys for schistosomiasis.

In all the isotopic and ELISA assays we have observed that control sera from uninfected tropical subjects give higher average values than sera from uninfected Europeans. At present we have no explanation for this but it may be relevant to note that tropical inhabitants often have anti-globulin activity in their sera, as well as elevated levels of other “auto-immune” indicators. The fact that sera from patients with sleeping sickness, in which such elevations are particularly common, often gave the highest cross-reactions supports this view. This emphasizes the point that for successful use in the tropics base-lines for ELISA or isotopic assays need to be established on the sera of uninfected people resident in that area. This is often very difficult especially for those diseases (e.g. malaria) which are widespread.

Overall the present results show that simple enzyme-
immunoassays are effective for the detection and measurement of antibody in several major parasitic diseases and suggests that they may be especially useful in disease surveillance in the future.

References


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