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Serodiagnosis of human cysticercosis by microplate enzyme-linked immunospecific assay (ELISA)*

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Summary

The micro-ELISA described in this report is simple and can give objective results for the serodiagnosis of human cysticercosis. The visual assessment of results was satisfactory. The ELISA results showed a remarkably good correlation with IHA. It gave good reproducibility. Cross-reactions were eliminated by using whole worm antigen instead of the cysticercus.

Key words: serology; ELISA; cysticercosis.

The application of the enzyme-linked immunospecific assay (ELISA) in the diagnosis of parasitic diseases represents a significant addition to existing serological tools.

In 1971, Van Weemen and Schurs (1971) in Holland and Engvall and Perlman (1972) in Sweden introduced the idea of using enzymes conjugated with antibody or with antigen to detect and measure antigens or antibodies, respectively. However, it was not until 1974 that these methods were used in parasitology. Encouraging results have been obtained with ELISA in such parasitic diseases as malaria (Voller et al., 1975a), Chagas' diseases (Voller et al., 1975b), trypanosomiasis (Voller et al., 1975c), toxoplasmosis (Voller et al., 1976), leishmaniasis (Hommel, 1976), schistosomiasis (Bout et al., 1975), trichinosis (Ruitenberg et al., 1974), and hydatidosis (Farag et al., 1975).

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

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The serological diagnosis of human cysticercosis is difficult, and is usually based on the critical use of one or more serological tests. Complement fixation, indirect hemagglutination, and double diffusion reactions have been used (Kagan and Norman, 1976). This is a report of our initial experience with ELISA and its potential in the serodiagnosis of human cysticercosis.

Material and methods

Forty-nine serum samples from Mexican patients suspected of having cysticercosis were used in this study. The serums were first tested using IHA according to the method of Kagan and Norman, 1976.

Two antigens were used: a delipidized saline extract of whole *Taenia solium* adult and its cysticercus. The antigens were standardized by box titration using a positive serum of known titer.

The ELISA procedure used in this study is the microadaptation of the technique as described by Walls (1976). Horseradish peroxidase (HRPO) was used as the marker enzyme and 5-aminosalicylic acid (5-ASA) as the substrate to visualize the reaction. The HRPO conjugate was prepared by Miss Bullock at the CDC parasitology laboratory according to the method of Nakane and Kawaoi (1974).

The procedure is briefly as follows:

1. The wells of polystyrene microtitration plates (Cooke No. 1-220-24A)* were sensitized with optimal dilutions of the antigens using 0.1 M carbonate buffer pH 9.6 as diluent. Whole worm antigen (WW) 1:300 and cysticercus antigen (Cy) 1:300 were used to sensitize the wells of the polystyrene plates. The plates were covered with plastic sealers (Cooke No. 1-220-30)* and incubated in a water bath at 37° C for 3 h. The antigen sensitized plates were stored in the refrigerator for future use.

2. The antigens were removed from the wells of the previously sensitized polystyrene microtitration plates by vigorous shaking and then washed 3 times with saline containing 0.05% Tween-20. Three min were allowed for each wash and the plates were shaken vigorously.

3. Serial dilutions of the test serums were prepared using PBS pH 7.2–7.3 with 0.05% Tween-20. 0.1 ml of test serum was added in each well and incubated in a water bath at 37° C for 30 min. The serums were shaken out and the plates washed as in #2.

4. Optimal dilution (1:800) of the HRPO conjugate in 0.1 ml amount was added in each well and the plates were again incubated in a water bath at 37° C for 30 min. After incubation, the conjugate was shaken out of the wells and the plates were washed as in #2.

5. The substrate (80 mg 5-ASA dissolved in 100 ml hot distilled water, 70° C) was prepared fresh each day. Immediately before using, the pH was adjusted to 6.0 with 1 N NaOH. One part of 0.05% hydrogen peroxide was then added to 9 parts of 5-ASA. 0.1 ml of the latter was added in each well and incubated at room temperature for 1 h. The results were read visually.

Results and observations

Of the 49 Serum samples from Mexican patients suspected of having cysticercosis, 36 or 73.47% reacted positively by IHA and 38 or 77.55% reacted positively by ELISA. The difference was not significant (χ^2 ldf = 0.128). The percentage of agreement between IHA and ELISA tests for cysticercosis was 95.56% (Table 1). Preliminary observations in malaria indicate that ELISA

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Table 1. Comparison of IHA and ELISA test for testing serum samples from humans suspected of cysticercosis

	Positive (%)	Negative
IHA	36 (73.47)	13
ELISA	38 (77.55)*	11

* χ^2 1 df = 0.128, not statistically significant
Percentage of agreement between IHA and ELISA tests = 95.56%

Table 2. Comparison of geometric mean titers of human serums positive for cysticercosis using IHA and ELISA test

	IHA	ELISA	
		cysticercus antigen	whole worm antigen
Geometric mean titer	382.45	522.43*	579.67**

* t = −6.176, statistically significant
** t = −7.22, statistically significant

results may correlate with those of hemagglutination tests better than with those of immunofluorescence (Voller et al., 1975a).

The reactions were read visually and the results were based on serial dilutions. End-point titers were determined. Positive ELISA result was indicated by a color reaction, ranging from light to deep violet. The higher the titer, the deeper the intensity of color change, since the degree of color change is proportional to the antibody concentration (Anonymus, 1976).

The IHA and ELISA geometric mean titers (GMT) of positive serums were compared. The GMT was higher using ELISA. With Cy antigen, the ELISA GMT was 522.43; significantly higher than IHA GMT of 382.45 (t = −6.176). The ELISA GMT using WW antigen was 579.67. This was significantly higher than when Cy antigen was used (t = −7.22) (Table 2).

Triplicate runs were conducted on positive serums and a known negative control to determine the reproducibility. Results of triplicate runs on 5 positive and 1 negative serums showed a high degree of reproducibility, with variations of 1 to 2 logs (Table 3).

The specificity of ELISA for cysticercosis was determined by testing serum samples positive for other parasitic infections (viz., echinococcosis, trichinosis, schistosomiasis, toxoplasmosis, and amoebiasis) against WW and Cy antigens. There was strong cross-reaction with echinococcosis using IHA. Eight out of the 9 echinococcosis positive serums reacted positively to cysticercosis by IHA. Using ELISA, a slight cross-reaction was observed (2 out of 15) with Cy antigen.

Table 3. Reproductibility of ELISA test on known cysticercosis positive human serum

Serum	Cysticercus			ELISA titer	
	IHA titer	Run		cysticercus antigen	whole worm antigen
74564	2048, 2048	1	+	2048	4096
		2	+	2048	4096
		3	+	1024	4096
69371	64, 256	1	+	1024	1024
		2	+	256	512
		3	+	256	512
17033	2048, 2048	1	+	1024	1024
		2	+	1024	1024
		3	+	4096	512
65717	512, 256	1	+	4096	2048
		2	+	4096	2048
		3	+	4096	1024
16045	128, 64	1	−	16	4
		2	−	16	2
		3	−	16	2
236769	−	1	−	−	−
		2	−	−	−
		3	−	−	−

Table 4. Specificity of ELISA test for cysticercosis and against other parasitic infections

Serum	Cysticercosis IHA	ELISA test	
		cysticercus antigen	whole worm antigen
Echinococcosis	8/8	2/15	0/15
Trichinosis	0/15	0/15	0/15
Schistosomiasis	0/15	2/15	0/15
Toxoplasmosis	0/15	0/15	0/15
Amoebiasis	0/15	0/15	0/15
Cysticercosis	36/49	38/49	38/49

The cross-reaction was eliminated when WW antigen was used (Table 4). The elimination of cross-reaction in ELISA could be partly attributed to the more selective absorption afforded perhaps by the polystyrene surface than by the surface of the tanned erythrocyte. It is not known what portion of the antigen is preferentially bound to the solid phase support (WHO, 1975).

It was observed that sensitized plates with the antigen inside the wells and then covered with plastic sealers to prevent evaporation remained reactive when kept in the refrigerator for as long as 8 weeks.

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