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Dot enzyme-linked immunosorbent assay (dot-ELISA) for schistosomiasis diagnosis using dacron as solid-phase

Dot-ELISA (dot enzyme-linked immunosorbent assay),utilizando o dacron como suporte sólido para o diagnóstico da esquistossomose

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Abstract *Dacron and nitrocellulose were evaluated as matrices for the dot enzyme linked immunosorbent assay (dot-ELISA) for schistosomiasis and compared to indirect immunofluorescence (IMF). Titration of sera from 18 schistosomiasis patients against soluble worm antigen preparation (SWAP) was carried out and sera from healthy individuals from non-endemic areas were used as controls. The IMF was less sensitive than the dot-ELISAs, although the difference was not statistically significant (p > 0.05). The dot-ELISA based on nitrocellulose was as sensitive as that using dacron. Stability did not differ between nitrocellulose and dacron. Specificity was lower when dacron was used than when nitrocellulose was used, although the difference was not statistically significant (p > 0.05). In conclusion, this work showed that nitrocellulose and dacron performed similarly in dot-ELISA, suggesting that they may be used alternatively in population surveillance in endemic areas.*

Key-words: *dot-ELISA. Dacron.* Schistosoma mansoni.

Resumo *O dacron e a nitrocelulose foram utilizados como matrizes para realização do dot-ELISA na esquistossomose e comparadas com a imunofluorescência indireta (IMF). A titulação dos soros de 18 pacientes esquistossomóticos foi feita, utilizando o antígeno solúvel de verme adulto (SWAP) e soro de pessoas normais não endêmicas foram usadas como controle. A IMF foi menos sensível do que os dot-ELISAs, apesar da diferença não ter sido estatisticamente significativa (p > 0,05). O dot-ELISA, utilizando a nitrocelulose foi tão sensível do que aquele utilizando o dacron como suporte. Não houve diferenças significativas entre os suportes em relação à estabilidade do antígeno. Entretanto, a especificidade, utilizando o dacron como suporte foi menor do que a nitrocelulose, apesar da diferença não ter sido estatisticamente significativa (p > 0,05). Em resumo, este trabalho mostrou que os resultados dos suportes utilizados em dot-ELISA para o diagnóstico da esquistossomose mansônica foram semelhantes, sugerindo que, o dacron também pode ser usado em levantamentos populacionais de areas endêmicas.*

Palavras-chaves: dot-ELISA. Dacron. Schistosoma mansoni.

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Immunodiagnostic techniques for schistosomiasis have been available since early this century[7 20] and have increased considerably in the last years due to a better understanding of the host-parasite interaction and the introduction of new concepts and methodologies[15].

The search for more sensitive, specific, practical and less expensive diagnostic tests has led to the development of a great number of immunological tests that could complement the limitations of the parasitological diagnosis[15]. Dot enzyme-linked immunosorbent assay (dot-ELISA) has been widely employed in several serologic tests because it does not need either sophisticated equipment or specialized personnel. Nitrocellulose is the most used matrix[10 11] due to the facility to handle it and the quality of the color developed[20], although it is very expensive. In 1987, Boctor et al[3], proposed the use of nitrocellulose strips as a matrix in dot-ELISAfor human schistosomiasis mansoni.

In our laboratory, dacron (polyethylene-terephthalate) has been used as solid phase in dot-ELISA for plague diagnosis[14 17]. This polyester has also been previously employed to immobilize enzymes yielding active derivatives[5 6 8 18 22].

In this work, soluble worm antigen preparation (SWAP) was covalently linked to dacron and dot-ELISA was tested for diagnosis of schistosomiasis. Comparative studies were carried out with dot-ELISA on nitrocellulose filters and indirect immunofluorescence assay (IMF).

MATERIALAND METHODS

Antigen. SWAP (soluble worm antigen preparation) from *Schistosoma mansoni* was obtained according to Boctor and Shaheen[2]. Sera from 18 patients presenting chronic schistosomiasis were obtained from Hospital das Clínicas, UFPE. Sera from 8 individuals from non-endemic areas and phosphate buffer saline (PBS) were used as negative controls. Sera from patients with Chagas' disease (3 patients), malaria (4 patients) and filariasis (3 patients) were used to test cross reactivity. Anti-human IgG labeled with horseradish peroxidase and 3,3-diaminobenzidine tetrahydrochloride (DAB) were purchased from Sigma, Chem. Co., St. Louis, MO. Tween 20 was acquired from Inlab. Dacron was produced by Rhodia do Brazil S.A. Nitrocellulose filter (0.22µm pore size) was from Millipore, Inc., Bedford, MA. All other reagents of analytical grade were obtained from Merck S.A., Brazil.

Dacron. Dacron plates were activated according to Oliveira et al[17] as modified by Montenegro et al[13].

Dot-ELISA. This technique was performed according to Pappas et al[18]. One ul of SWAP antigen (0.25µg/µl) was spotted onto the azida-dacron plates and nitrocellulose filters and kept at 37ºC for 30min to dry. The antigen-dacron plates and antigen-nitrocellulose preparations were introduced into the wells of a Nunc multidish plate and stored at 4ºC overnight. Protein concentration was evaluated according to Bradford et al (1976)[4]. The blocking substance used was 30% (w/v) non-fat instant milk (Fleichmann and Royal Ltda., Brazil) solution (0.4ml) prepared in PBS, pH 7.2, containing 0.05% (v/v) Tween-20 (PBS-Tween 20) and kept for 15min with gentle stirring. The blocking solution was aspirated out and the plates were washed three times with PBS-Tween 20 with shaking, during 1min each and the last wash was preceeded by a 10 min incubation without stirring. The serum samples and controls were serially diluted in PBS-Tween 20 from 1:16 to 1:65,536 and introduced into the wells containing the blocked antigen-dacron plates or antigen-nitrocellulose filters. The plates and filters were shaken for 1min at 25ºC, incubated for 30min and the washing procedure was carried out as described for the blocking. Anti-rabbit IgG peroxidase conjugate (IgG-HRP) was diluted 1:1,000 in PBS and 0.6ml was pipetted into each well. The plate was shaken for 1 min and incubated for 30min. The plates and filters were washed five times as described before. The development of the spot was perfomed according to Montenegro et al (1991)[14].

Indirect Immunofluorescence assay (IMF). This technique was undertaken according to Helden et al (1975)[9] and cercariae obtained from infected *Biomphalaria glabrata* were used as antigen.

Temporal and thermal stabilities of the antigen. Antigen-dacron plates and antigen-nitrocellulose filters were stored at 28ºC, 4ºC and -20ºC for a time ranging from 7 to 90 days. Then, dot-ELISA was carried out according to the methodology described using positive sera in PBS-Tween and anti IgG-HRP conjugate in PBS at dilutions of 1:100 and 1:1,000, respectively.

Statistical analysis. A 95% confidence qui-square test (χ^2) was used for comparison between the sensitivities and especificities of different assays.

RESULTS

The titration of sera obtained from schistosomiasis patients by using IMF and dot-ELISA on nitrocellulose and dacron is shown in Table 1. Two patients (numbers 6 and 18) were negative by using IMF tests and positive by using dot-ELISAs. In the control group (non-endemic area), only one individual showed a spot at a dilution of 1:64 when nitrocellulose was used as matrix. When dacron was used as matrix, spots were shown at dilutions of 1:32 (three persons) and 1:256 (one person). No positive reaction was observed using PBS as negative control. Based upon these results we established a cut-off point of 1:256 for both matrices. All sera from *S. mansoni* patients reacted positively in both nitrocellulose and dacron, resulting in a sensitivity of 100%.

All malaria sera (n = 4) and one out of 3 filariasis sera were reactive at 1:512 dilution and were considered cross-reactions using dacron as solid phase while no sera from Chagas' disease patients (n = 3) were reactive. Using nitrocelulose as solid phase no cross-reactivity was observed. The specificity for nitrocellulose and dacron was 100% and 87.5%, respectively, although the difference was not statistically significant ($p > 0.05$).

Table 1 - Titration of sera from schistosomiasis patients by using indirect immunofluorescence assay and dot-ELISAs on dacron and nitrocellulose (reciprocal titers).

		Dot-ELISA	
Patients	IMF	Dacron	Nitrocellulose
1	256	4,096	8,196
2	256	1,024	4,096
3	256	2,048	4,096
4	128	4,096	2,048
5	512	8,192	16,384
6	neg	1,024	4,096
7	128	4,096	2,048
8	128	4,096	4,096
9	128	512	1,024
10	128	2,048	8,192
11	128	1,024	1,024
12	128	1,024	2,048
13	256	4,096	8,192
14	256	512	1,024
15	256	2,048	8,192
16	128	2,048	2,048
17	128	512	1,024
18	neg	8,192	8,192

neg = negative

The IMF using as cut-off point a 1:64 dilution was less sensitive (88.9%) than the dot-ELISAs, although the difference was not statistically significant ($p > 0.05$).

Dot-ELISA carried out on antigen-dacron plates stored at -20ºC at 60 days still showed the same spot intensity as those recently prepared. The antigen-dacron plates stored at 4ºC presented weaker reactions at 15, 30 and 60 days and stored at 28ºC for 60 days it was not suitable for diagnosis. The results on stability were not so different regarding nitrocellulose dot-ELISA (Table 2).

Table 2 - Determination of antigen stability on dacron and nitrocellulose in relation to time and temperature.

Days	Nitrocellulose			Dacron		
	4ºC	28ºC	-20ºC	4ºC	28ºC	-20ºC
7	++	++	++	++	++	++
15	++	++	++	+	+	++
30	+	+	+	+	+/-	++
60	+	+	+	+	-	++

DISCUSSION

We have previously studied the dacron as a solid matrix in a dot-ELISA with plague bacillus antigen and found results as good as the nitrocellulose that is the most common matrix used[1 14]. Nitrocellulose is the most widely used matrix but it is very expensive and the dacron has the advantage that can be obtained at very low cost. In this study, we evaluated the perfomance of dacron and a dot-ELISAto detect antibodies to schistosome worm antigen.

The only false positive reaction using sera from non-endemic control group and the cross-reactions with malaria and one filaria sera was in dacron matrix. In the first case this could be attributed to the fact that these individuals may have been parasited by several others helminth species and could have cross-reactions in the dot-ELISA[3]. We have no explanation for the positive reaction of sera from malaria patients; we do not know whether they had ever been in an endemic area for schistosomiasis. On the other hand, it is well known that, cross reaction between *Schistosoma* and filaria antigens can be observed regularly in ELISA[14].

The present results demonstrate that nitrocellulose and dacron perform similarly in dot-ELISA. Thus, taking into account that dacron can be obtained at very low cost, it is easily available, presents the same sensitivity of nitrocellulose (although our study is preliminary) and covalently linked antigens are stable for long periods, we suggest that this material could be used as an alternative matrix in surveillance of populations in endemic areas. According to Mott and Dixon (1982)[16], one point that has been generally accepted in immunological tests is that improvement in the specificity of immunodiagnostic test will depend on the availability of pure antigens. We are currently assaying the dacron matrix with purified antigens and increasing the size of the sample studied.

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