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ENHANCED SENSITIVITY OF DOT-IMMUNOBINDING ASSAYS FOR THE DETECTION OF ANTIBODIES IN MICE BY THE USE OF MILD DETERGENT EXTRACTED *TRYPANOSOMA CRUZI* ANTIGENS

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DE GASPARI E. N.; STOLF A.M.S. & ABRAHAMSOHN, I.A. — Enhanced sensitivity of dot-immunobinding assays for the detection of antibodies in mice by the use of mild detergent extracted *Trypanosoma cruzi* antigens. *Rev. Inst. Adolfo Lutz*, 50(1/2): 231-233, 1990.

ABSTRACT: A method for a rapid and accurate demonstration of antibodies to *Trypanosoma cruzi* by using nitrocellulose paper strips with antigens of the parasite was developed and evaluated. We report herein additional advantages of its use for the detection of specific antibody to *Trypanosoma cruzi* in the sera of mice. Increased sensibility without increase in the background was achieved using parasite antigens obtained by mild non-ionic detergent extraction as compared to antigens extracted by alkaline digestion.

DESCRIPTORS: *Trypanosoma cruzi;* Dot-immunobinding assays; epimastigotes; trypomastigotes; non-ionic detergent.

The dot-immunobinding assay (DI) has already proved itself to be a convenient and rapid serodiagnostic technique for the detection of antibodies against protozoa in human serum[1,2,3,4]. We report, herein, additional advantages of its use for the detection of specific antibody to *Trypanosoma cruzi* in the sera of mice. Increased sensitivity without increase in the background was achieved using parasite antigens obtained by mild non-ionic detergent extraction as compared to antigens extracted by alkaline digestion. Detergent extraction ensures a better preservation of antigenic molecules. Although the antigens thus prepared do not adhere to plastic surfaces and are therefore unsuitable for ELISA assays, they can be diretly applied on nitrocellulose sheets without any further treatment.

C57B1/10J (B10), A/Sn, Balb/c, CBA and outbred albino mice infected (i.p) with subletal inocula of *T. cruzi* (Y strain) blood forms. At different days after infection 100 µl samples of blood were collected from the ophtalmic plexus. Each pool of sera was obtained from 15 to 25 mice. Epimastigotes were grown in liver infusion tryptose medium and washed three times by centrifugation (600 g, 10 min., 4°C) in cold sterile 0.01M phosphate buffered saline (PBS). Tissue culture trypomastigotes grown in LLC-MK2 cell cultures were harvested from the supernatant and washed three times (1000 g, 10 min., 4°C) in Dulbecco's Modified Eagle Medium (DMEM, Flow Labs. Inc., Rockville, MD). Alkaline digestion (0.15M NaOH) of pelleted epimastigotes was carried out exactly as described by

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HOSHINO-SHIMIZU[6]. Detergent extraction was carried out by suspending 2 X 10^7 pelleted epimastigotes or trypomastigotes in 300 µl of 0.65% (v/v) Nonidet- P40 (NP-40, Shell Química S.A., S. Paulo, Brasil) in TBS (50 mM NaCl buffered with Tris (hydroxymethyl) aminomethane 2 mM to pH. 7.5). The parasites were incubated in NP-40 for 5 min at room temperature (RT) and kept frozen at - 20°C. The three antigen preparations obtained: EPI-alkaline, EPI-NP40, and Trypo-NP40 were titrated "en bloc" against positive and negative reference sera. One microliter samples were deposited on nitrocellulose membrane strips (Bio Rad Labs. Inc., Richmond, CA) and dried at 37°C . The actual amount of antigen in 1 µl corresponded to: 1.2 x 10^5 parasites for EPI-alkaline, 6.2 x 10^4 parasites for EPI-NP40 and 3.1 x 10^4 parasites for Trypo-NP40. All the next procedures were carried out at RT and all working solutions were made in TBS.

The DI assay was performed as described by LISSALDO[2]. Briefly, the nitrocellulose sheets were blocked with 5% (w/v) skimmed milk (Molico, Nestlé do Brasil, MG, Brasil) for 2h and rinsed twice in TBS. The dilutions of the serum samples were done in 96 well dilution plates to wich the antigen dotted strips were firmly clamped and incubated for 1h on a rocking platform. The strips were rinsed in 0.05% (v/v) NP40 and covered with peroxidase labelled goat anti-mouse IgG (Cappel, Cooper Biomedical Inc., Malvern, PA) for 1h titre 1/500 (v/v). After rinsing in PBS, the strips were incubated in the substratre (titre 1/500 v/v) chromogen solution (Diamino-benzidine 0.01% (w/v) and 1/400 (v/v) dilution of 30% (v/v) H_2O_2, in PBS). The strips were rinsed, dried and read by visual inspection. The last serum dilution resulting in a yet discernible brown spot was considered its titre. The titres obtained for the pools of sera tested against the three different antigens were compared using the Sign test[5]. Normal mouse sera (pooled) from any of the strains used were consistently negative in dilutions as low as 1/5.

The use of NP40 extracted antigen led to an increased of sensitivity of the order of at least twice the $\log^2$ titres obtained for the same sera tested against alkaline extracted antigen (Table). Thus, the better conservation of antigen structure in the presence of mild detergent extraction is a

TABELA

Dot - Immunobinding assay of antibody to alkaline and detergent extracted T. Cruzi antigens in infected mice.

		Reciprocal titres of sera tested to antigens		
Days PI	Mouse strain	Epi-NPA40*	Epi-alkaline+	Trypo-NP40‡
8	A/Sn	20	5	10
	B/10	20	5	10
	Balb/c	20	20	10
	A/Sn	80	40	160
11	B10	40	20	80
	Balb/c	40	10	80
14	CBA	160	40	320
	A/Sn	160	20	160
18	B10	160/160/160§	20/20/20§	320/320/320§
	CBA	80	20	160
	A/SN	320/320/640§	40/40/40§	640/320/640§
	B10	320/320/640/640§	80/40/160/160§	640/320/640/640§
45-65 (Chronic phase)	Balb/c	320/640§	40/160§	320/640§
	Outbred	640	10	640

* Epimastigote antigen obtained by detergent extraction; +Epimastigote antigen obtained by alkaline extraction; ‡ Tissue culture trypomastigote antigen obtained by detergent extraction. § Each value corresponds to independent pools of sera tested, which were tabulated in the same order in any of the three columns presented above. Statistic analysis: Epi-NP40 vs. Epi-alkaline: $p < 0.001$ for sera from 8 to 18 days PI; $p < 0.005$ for chronic phase sera. Epi-NP40 vs. Trypo-NP40: $p > 0.1$.

major advantage of the dot assay. Moreover, the dot assay is more economical since even minute amounts of antigen are totally bound to the membrane. This investigation was supported by FAPESP and CNPq-PIDE VI to I.A.A. and to A.M.S.S. E.N. De Gaspari acknowledges her fellowship from FAPESP. The authors are indebted to Drs. Yara Juliano and Neil Ferreira Novo from the Biostatistics Division of the Dept. de Medicina Preventiva, Escola Paulista de Medicina, for statistical analysis.

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DE GASPARI E.N., STOLF A.M.S & ABRAHAMSOHN, I.A. — A utilização de detergente na extração de antígenos de *Trypanosoma cruzi* aumentou a sensibilidade da reação imunoenzimática (Dot-immunobinding assay), para a quantificação de anticorpos de camundongos. *Rev. Inst. Adolfo. Lutz*, 50(1/2): 231-233, 1990.

RESUMO: Um método rápido e preciso para a demonstração de anticorpos anti-*Trypanosoma cruzi* usando papel de nitrocelulose com antígenos do parasito foi padronizado e avaliado Mostramos neste estudo as vantagens de sua utilização na detecção de anticorpos específicos no soro de camundongos. Observamos aumento da sensibilidade sem aumento da inespecificidade utilizando-se antígenos do parasito tratado com detergente não iônico.

DESCRITORES: *Trypanosoma cruzi*, reação imunoenzimática, epimastigota, tripomastigota, detergente não iônico.

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