Serodiagnosis of *Trypanosoma cruzi* Infection Using the New Particle Gel Immunoassay - ID-PaGIA Chagas

Ana Rabello/+, Alejandro O Luquetti*, Eliana F Moreira, Maria de Fátima Gadelha***, José Alisson dos Santos**** Laércio de Melo****, Peter Schwind*****

Centro de Pesquisas René Rachou-Fiocruz, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil *Universidade de Goiás, Goiânia, GO, Brasil **Hemocentro de Pernambuco, Recife, PE, Brasil ***Fundação Ezequiel Dias, Belo Horizonte, MG, Brasil ****DiaMed Brasil, Belo Horizonte, MG, Brasil *****DiaMed AG-Cressier sur Morat, Switzerland

The ID-Chagas test is a particle gel immunoassay (PaGIA). Red coloured particles are sensitised with three different synthetic peptides representing antigen sequences of Trypanosoma cruzi: *Ag2, TcD and TcE. When these particles are mixed with serum containing specific antibodies, they agglutinate. The reaction mixture is centrifuged through a gel filtration matrix allowing free agglutinated particles to remain trapped on the top or distributed within the gel. The result can be read visually. In order to investigate the ability of the ID-PaGIA to discriminate negative and positive sera, 111 negative and 119 positive, collected in four different Brazilian institutions, were tested by each of the participants. All sera were previously classified as positive or negative according to results obtained with three conventional tests (indirect immunofluorescence, indirect hemaglutination, and enzime linked immunosorbent assay). Sensitivity rates of ID-PaGIA varied from 95.7% to 97.4% with mean sensitivity of 96.8% and specificity rates varied from 93.8 to 98.8% with mean specificity of 94.6%. The overall Kappa test was 0.94. The assay presents as advantages the simplicity of operation and the reaction time of 20 min. In this study, ID-PaGIA showed to be highly sensitive and specific.*

Key words: Chagas' disease - immunodiagnosis - serological tests-particle gel immunoassay

The present epidemiological data concerning the burden of Chagas' disease claim for new strategies for disease management and control. According to the World Health Organization (WHO 1997), 16-18 million people are currently infected, and 25% of the total population in Central and South America are at risk (TDR, Progress Report 95-96). The most important route of transmission of this endemic disease caused by the protozoan parasite *Trypanosoma cruzi* is by contact of a human being with an infected triatomine bug. Transfusional transmission is considered the second main route of infection and in some endemic regions, where vectorial transmission is under control or in countries where triatomine vectors are not settled, blood transfusion becomes the most important mechanism of disease transmission (Moncayo 1992, Dias & Coura 1997). Screening of infected blood, in blood banks, aiming the interruption of the transfusional transmission of this disease represents an important measure among the control priorities (Schmunis 1991). Interruption of transmission in Southern Cone, Argentina, Brazil, Chile and Uruguay, due to initiatives of coverage of blood banks by screening, is expected in 1998. The target in Colombia, Ecuador and Peru was to implement blood screening by 1997. Emphasis on blood bank is being ensured in Costa Rica, El Salvador, Guatemala, Mexico, Nicaragua, Panama and Honduras. Screening is now mandatory in Bolivia, where prevalence of *T. cruzi* infected blood in blood banks has been as high as 63% (Schmunis 1985).

In chronic stage of the infection, detection of the parasite in blood by direct parasitological examination, hemoculture or xenodiagnosis is highly specific, but sensitivity varies from 34% to 85% depending on technical conditions (Cedillos et al. 1970, 1982, Cerisola et al. 1971, Luz et al. 1994). Thus, detection of serum antibodies to *T. cruzi* antigens has been the main method for the diagnosis of Chagas' disease.

Among several serological tests available the most commonly used are the indirect hemaglutination assay (IHA), the indirect immunofluorescent test (IIFT), the direct agglutination (DA)

+Corresponding author. Fax: +55-31-295.3115. E-mail: ana@netra.cpqrr.fiocruz.br
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and the enzyme linked immunosorbent assay (ELISA). Most of these serological tests are based on reagents prepared from whole parasites or complex mixtures of antigens; technical procedures may differ from one laboratory to the other, so variable results are obtained.

Different defined *T. cruzi* antigens, purified proteins from parasite lysates and recombinant antigens have been produced since the late 80s. Eleven of them showed sensitivity varying from 82 to 100% and specificity varying from 83 to 100% when tested in a double blind multicentric study (Moncayo & Luquetti 1990).

The development of diagnostic methods that combine reliable antigens, as synthetic peptides, with a simple and reproducible assay is highly desirable for endemic disease control programs. In this work, a novel immunodiagnostic assay for Chagas' disease immunodiagnosis, the ID-Chagas antibody test is described and its performance is compared to the conventional serology.

MATERIALS AND METHODS

Sample size - In order to investigate the ability of the ID-PaGIA Chagas test to discriminate negative and positive sera, a sample size of 100 negative and 100 positive serum samples was required using as parameters a minimum Kappa agreement of 0.80 and an expected tests concordance of 90% (Landis & Koch 1977).

Two hundred and thirty samples, 119 positive and 111 negative, collected in four different Brazilian institutions were interchanged and all tested by each of the participants of the respective institution: (i) Centro de Pesquisas René Rachou-Fiocruz (positive sera from Bambuí, endemic area of the State of Minas Gerais, negative sera from Ibituruna, a non-endemic area for Chagas' disease); (ii) Universidade Federal de Goiás (frozen samples from the Laboratório de Pesquisa da Doença de Chagas, Faculdade de Medicina, that is the reference for local blood banks and patients care units); (iii) Hemocentro de Pernambuco (samples recently collected from patients living in Recife and from blood donors) and (iv) Fundação Ezequiel Dias, Funed, in Belo Horizonte (sera from blood donors and health basic care in Minas Gerais). Negative samples were all from normal individuals.

Conventional serological tests - Three serological tests (IIFT, IHA and ELISA) were performed. The IIFT was performed according to Camargo and Hoshino-Shimizu (1974). *T. cruzi* epimastigotes were used as antigen and fluorescein isothiocyanate conjugated sheep anti-human immunoglobulin G, (Fluoline G, Biolab Diagnóstica SA, Rio de Janeiro) was used as the second antibody. The Chagas HAI-Immunoserum kit (Immunoserum-Tecnologia Imunológica Indústria e Comércio, São Paulo, Brazil) was used for IHA and the ELISA-Hemobio Chagas (Embrabio, Empresa Brasileira de Biotecnologia, São Paulo, Brazil) or Chagnostika kit (Organon Teknika, São Paulo, Brazil) were used for the ELISA test.

All sera included were split into two groups, according to the serological results obtained: those belonging to non infected individuals and those from infected people. Conventional serology that presented discordant results was confirmed by the Laboratory of Chagas' disease from the University of Goiás for all sera that presented discrepant results.

ID-Chagas antibody test - The ID-Chagas antibody test is a particle gel immunoassay (PaGIA). The ID Micro Typing System (gel test) which is a well known system for immuno-hematology laboratorial procedures, monitors hemagglutination reactions by the focusing and spatial separation of erythrocytes within a gel matrix. In the PaGIA system (Fig. 1) erythrocytes are replaced by coloured polymer particles coated with the target antigens. When antigen coated particles are mixed with serum containing specific antibodies, particles do agglutinate. The reaction mixture is centrifuged through a gel filtration matrix allowing free non-agglutinated particles to deposit on the bottom of the microtube while agglutinated particles remain trapped on top or distributed within the gel. The result can be read visually.

Particle preparation - The standard PaGIA beads consist of red coloured particles which serve as a universal carrier (Hobbs et al., manuscript in preparation). Beads were coated with an optimised mixture of three different synthetic peptides representing antigenic sequences from *T. cruzi* trypomastigote antigens: Ag2, TcD and TcE previously described (Peralta et al. 1994, Reed 1996). The synthetic peptide-coated solid phase beads were stored refrigerated as a ready-to-use 0.15% solids (w/v) suspension. The same batch of sensitized beads was used in all institutions.

ID-Cards - The ID-Micro Typing System uses specially designed ID-Cards. Each card has six reaction chambers containing a buffered Sephacryl gel matrix.

Test procedure - After appropriate identification of serum the aluminium foil that covers the ID-Cards is removed and the ID-Cards are placed vertically in the cards holders. The polymer particles reagent tube is mixed in a vortex thoroughly for 5 sec and 25 µl of plasma or serum are then added into the particle suspension in the microtube. Reactants are mixed by aspirating and expelling one volume of particles/serum mixture within the reaction chamber. The ID-Cards are incubated for

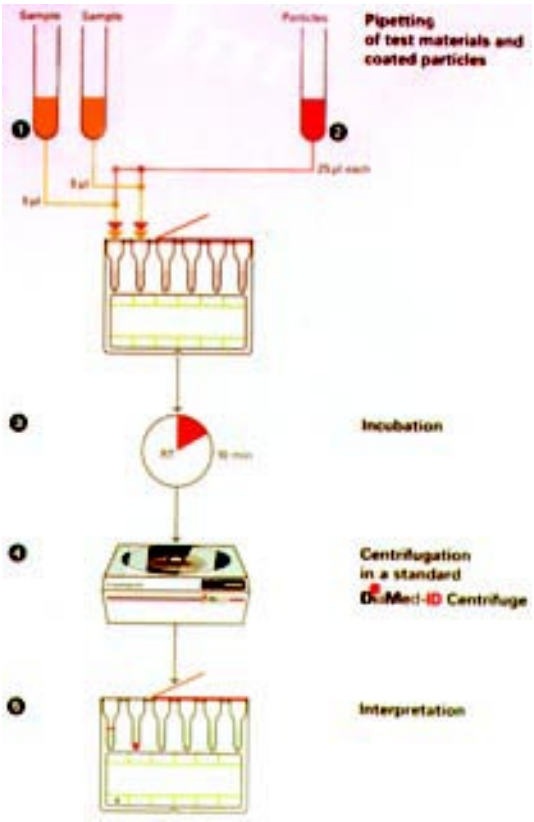

Fig. 1: particle gel immunoassay methodology.

10 min at room temperature. After incubation, ID-Cards are centrifuged for 10 min in a special ID-Centrifuge and reaction are read and recorded.

Interpretation of the results - Positive results, which indicate the presence of detectable levels of anti-*T. cruzi* antibodies are typically illustrated by the presence of a tight red line on the top of the gel surface or by the presence of agglutinated beads distributed within the gel matrix. Negative results were illustrated by the presence of a tight pellet of particles at the bottom of the microtube following centrifugation with no agglutinated particles present within or in the top of the gel (Fig. 2).

Results of ID-PaGIA Chagas were considered positive or negative by independent visual interpretation agreement of three technicians not aware of conventional serology classification.

Statistical analysis - Sensitivity and specificity of the ID-Chagas antibody test were calculated based on conventional serology criteria: all three positive or all three negative conventional serology tests. Kappa agreement was calculated to evaluate agreement between conventional serology and ID-Chagas test (Landis & Koch 1977).

RESULTS

Sensitivity rates varied from 95.7% to 97.4% with mean sensitivity of 96.8% and specificity rates varied from 93.8 to 98.8% with mean specificity of 94.6%. The overall Kappa test was 0.94. Com-

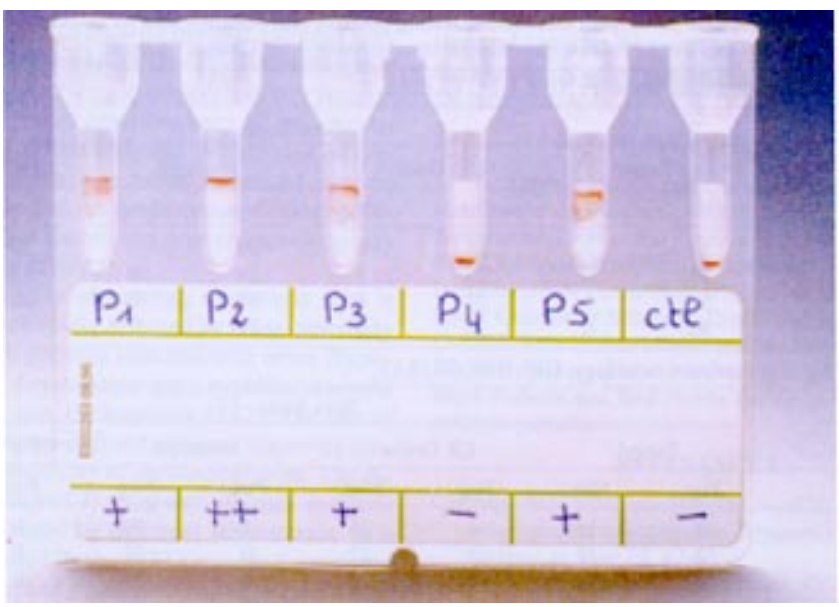

Fig. 2: interpretation of the ID-particle gel immunoassay Chagas' antibody test. P1, P2, P3 and P5: positive results. P4: negative result. Ctl: negative control.

parisons of results performed by each of the institutions can be seen in Table I.

The grouped results from each institution are shown in Table II. Inter-laboratory reproducibility was high since of 476 tests done with the 119 positive sera, six sera gave false negative results by all institutions and of 444 tests done with the 111 negative sera, 11 sera gave false positive results by all institutions.

After appropriate training of the technicians visual interpretation was easy and fast. Only seven sera out of the 230 were differently interpreted by

TABLE I

Sensitivity, specificity and Kappa test of ID-PaGIA (particle gel immunoassay) as compared to conventional serology for Chagas' disease

Positive sera by conventional serology (IIF, IHA, ELISA)			
	ID - PaGIA		
Tested by	Positive	Negative	Sensitivity (%)
Funed (n = 119)	115	4	96.6
UF Goiás (n = 119)	116	3	97.4
Hemope (n = 119)	114	5	95.8
CPq René Rachou (n = 119)	116	3	97.4
Negative sera by conventional serology (IIF, IHA, ELISA)			
	ID-PaGIA		
Tested by	Positive	Negative	Specificity (%)
Funed (n = 111)	9	102	91.9
UF Goiás (n = 111)	6	105	94.6
Hemope (n = 111)	5	106	95.5
CPq René Rachou (n = 111)	11	100	90.1

Overall results: sensitivity = 96.8 %; specificity = 94.6 %; Kappa test = 0.94; Funed: Fundação Ezequiel Dias; UF Goiás: Universidade Federal de Goiás; Hemope: Hemocentro de Pernambuco; Cpq René Rachou: Centro de Pesquisas René Rachou-Fiocruz.

TABLE II

Agreement among the four participant institutions testing positive and negative sera by conventional serology and by ID-PaGIA (particle gel immunoassay) for Chagas' disease

Positive sera by conventional serology (IIF, IHA, ELISA)								
	Sera from							
	Funed		UF Goiás		Hemope		CPq René Rachou	
Tested by	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Funed	30	0	26	4	29	0	30	0
UF Goiás	30	0	27	3	29	0	30	0
Hemope	30	0	25	5	29	0	30	0
CPq René Rachou	30	0	27	3	29	0	30	0
Negative sera by conventional serology (IIF, IHA, ELISA)								
	Sera from							
	Funed		UF Goiás		Hemope		CPq René Rachou	
Tested by	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Funed	4	26	4	26	1	20	0	30
UF Goiás	3	27	3	27	0	21	0	30
Hemope	1	29	4	26	0	21	0	30
CPq René Rachou	4	26	4	26	2	19	1	29

Funed: Fundação Ezequiel Dias; UF Goiás: Universidade Federal de Goiás; Hemope: Hemocentro de Pernambuco; Cpq René Rachou: Centro de Pesquisas René Rachou-Fiocruz.

one of the three technicians which gives a percentual agreement of 96.9%. From these seven sera, five were considered positive by two and doubtful by one technician. The other two sera were considered positive by two technicians and negative by the third one.

DISCUSSION

In this study, the new PaGIA - ID-PaGIA Chagas - has been compared to three conventional immunoassays for the Chagas' disease serodiagnosis. Although high sensitivity and specificity have been found for each of the institutions involved, differences among them could be observed. A higher frequency of false positive and false negative results occurred in Goiás. Several variables that may have influenced the test performance cannot be ruled out, for instance, false-negative results may be related to parasite strains and/or individual host immune response. Since detailed epidemiological and clinical analyses were beyond the scope of this work other studies are required to investigate the regional differences.

Criteria of positivity was based on three serological positive and negative sera in order to have the best attainable serological definition. Further studies will address the comparison of the ID-PaGIA Chagas with each of the three conventional serological tests.

Different circumstances make the standardization of Chagas' disease serodiagnosis difficult. Ideally, the more efficient tests may result from the standardization of pure immunodominant fractions of *T. cruzi* antigens to replace the complex extracts of the parasite. Sensitivity with defined antigens may be improved by the association of different antigenic sites selected according to complementary reactivity and non-specific reactions may be avoided by the use of appropriate antigens. With the use of synthetic peptides batch to batch variations may be largely reduced and the development of simple format methods that lessens test steps and handling may offer increased safety.

The specificity of existing diagnostic tests is known to be adversely affected by cross-reactivity with sera from patients infected with other *Trypanosoma* spp., *Leishmania* spp., syphilis, *Amoeba* spp., *Giardia* spp. (Schottelius 1982). The use of purified and better-defined antigens improves sensitivity and specificity of serological tests. The diagnostic efficacy of *T. cruzi* recombinant antigens has been evaluated by different laboratories. In a study coordinated by the WHO specificity and sensitivity of 10 out of 17 recombinant antigens tested equal or higher than 0.80 with sensitivity ranging from 95 to 100% and specificity ranging from 86 to 100% (Schmunis 1991). Although several recombinant antigens displayed high sensitivity and specificity some of them were unable to detect anti-*T. cruzi* antibodies in four out of 148 chronic chagasic patients (Pan et al. 1992).

The cross reactivity of TcD and PEP2 peptides has been studied by Peralta et al. (1994). The specificity was 99% in an ELISA test using the association of these two antigens tested for samples from patients with cutaneous leishmaniasis, visceral leishmaniasis, leprosy, tuberculosis and for samples from healthy individuals.

In the ID-Chagas antibody test, the use of well defined antigens in the form of highly purified synthetic peptides serves to minimise such cross-reactivity. Cross-reactivity with visceral leishmaniasis was observed only in three of 25 sera obtained from patients with visceral leishmaniasis (Schwind, pers. commun.).

The ID-PaGIA format presents a fast and simple alternative to ELISA because only two sequential pipetting steps are needed as it is devoid of multiple reagent addition and wash steps intrinsic to the ELISA technique. The exposure to potentially infectious material inherent to all previously described techniques is significantly reduced as the technique requires only the single addition of an undiluted sample along with the non-infectious particle reagent. Thereafter, all reagents remain within the test card which may be resealed to completely enclose the samples. The cost of the test is comparable to an ELISA.

In this study, direct comparisons between the ID-PaGIA and conventional serology have shown that the ID-Chagas antibody test is highly sensitive and specific. The assay presents as advantages the simplicity of operation and the reaction time of 20 min.

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