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USE OF *TRYPANOSOMA CRUZI* DEFINED PROTEINS FOR DIAGNOSIS – MULTICENTRE TRIAL(*) SEROLOGICAL AND TECHNICAL ASPECTS

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INTRODUCTION

Due to low parasitemia, the diagnosis of Chagas' disease during the chronic phase of infection is performed mainly by indirect methods, i.e., search for antibodies against *Trypanosoma cruzi* in sera.

Although there are several serological techniques available, cross-reactivity, standardization of reagents, and diversity of technical procedures, are still problems to be solved. Considerable variation in the reproducibility and reliability of results obtained from different research Labs has been often reported (Prata et al., 1976; Camargo et al., 1986). An even worse situation would be expected from routine Labs, which usually perform these tests in different situations and for several purposes, where the conditions are far from ideal.

Several studies have been performed by different groups in order to standardize currently available techniques (Camargo et al., 1986; Almeida & Fife, 1976) but some of the problems remain unsolved. For this reason, there is a need to improve the techniques available with the introduction of purified antigens and, recently, to study the potential use of recombinant proteins, which have been suggested to increase sensitivity and specificity of the serological tests.

In order to evaluate the potential use of these recombinant proteins and synthetic antigens the TDR Joint Steering Committee on Chagas' disease at its meeting in June 1989, proposed a multicentre study including different laboratories (TDR, 1989). The Lab. of Chagas' disease, of the Federal University of Goiás, Brazil, was selected to coordinate the study, together with the Secretariat of TDR Joint Steering Committee on Chagas' disease.

THE PROBLEM OF SEROLOGICAL DIAGNOSIS IN CHAGAS' DISEASE

1) *Techniques currently available* – The ideal serological technique should be easy to perform, quick in reading results, reliable, and cheap. Sensitivity and specificty should be high, close to 100%.

The serological diagnosis of Chagas' disease was described right after the discovery of the disease. Guerreiro and Machado, in 1913 described the Complement Fixation reaction, which was routinely used as the single technique in many laboratories, until recently (Guerreiro & Machado, 1913). It is a tedious test, which involves several reagents that require strict quality control, difficult to obtain in routine Labs.

In the past 20 years, Knierim & Saavedra (1966); Cerisola et al. (1967) and others described and employed haemagglutination tests, which are currently used with success. Indirect haemagglutination (IHA) is the easiest of the techniques available, with high specificity but its sensitivity is lower than other commonly employed techniques, so it is not appropriate

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as a single diagnostic test. It is also expensive if obtained in the market as kits.

Indirect Immunofluorescence (IIF) has been used for the serological diagnosis of Chagas' disease by Camargo and others since 1966 and is still employed extensively, with success (Camargo, 1966). Among other advantages, it is cheaper than IHA, and allows to process a great number of samples simultaneously. Nevertheless the reading is subjective, mainly in borderline cases, and its specificity is not high, giving cross reaction in low titers with several diseases, mainly visceral leishmaniasis.

A good combination has been the use of IHA and IIF, when we may compensate the relatively low sensitivity of IHA with the high sensitivity of IIF, and the rather low specificity of IIF with the higher specificity of IHA.

Direct agglutination (DA) has been a successful technique, mainly after the treatment of sera with 2 mercaptoethanol (DA2ME) (Storni et al., 1975), which increases its specificity. We employ it routinely and find it very useful, because of its higher sensitivity. Nevertheless, it is not employed in the majority of Labs, because it is as expensive as IHA, has a further technical step (2ME), and is not so easy to read because of the white colour of trypsinized epimastigotes.

Other tested techniques as Latex coated particles, yielded poor results, mainly due to low specificity and sensitivity. Radioimunoassays have been described, but they may be not suitable for routine Labs.

Voller's introduction of ELISA in 1975 (Voller et al., 1975) has been a good improvement for *T. cruzi* infection diagnosis, but not used as routine in non-specialized Labs, mainly due to the many steps involved. It proved to have high sensitivity, but in our hands, its specificity with crude antigens is far from ideal, mainly by an array of borderline results. The cut-off point should be adjusted precisely for each plate and each experiment with which the borderline results may be handled better (Zicker et al., 1990). Also, differences in optical density are detected depending on the position of the well under reading, so duplicates of each sera are mandatory, preferentially apart away in the plate. It is necessary also to include several controls for each plate, with which the actual number of sera to be tested decreases, increasing costs per tested sample.

Other techniques, as complement mediated lysis (Krettli et al., 1982) are time consuming, requiring the use of alive parasites (which makes them dangerous for routine work), and their results have had similar problems to the conventional techniques (Luquetti et al., 1987).

2) *Different situations in the serological diagnosis of* T. cruzi *infection* – In medical practice, we have different situations in which serodiagnosis for Chagas' disease is required. Each one of the following situations need several adjustments, mainly to employ different cut-off levels and the use of a particular set of techniques.

a) Serological confirmation of a clinically suspected chagasic patient – In this case, a physician requires from the Lab., a serological confirmation of the etiology for a patient who may have epidemiology and clinical manifestations of the disease. In this situation we require high specificity and sensitivity, which may be obtained by the combination of two of the techniques referred above. A false negative result may lead to a wrong etiological diagnosis. Perhaps a false positive result will be even worst, because it will rotulate the patient as "chagasic", creating a social problem in the absence of infection (Camargo & Takeda, 1979).

b) Screening of Chagas' disease in blood donors – This is a very different situation, in which we require the maximum sensitivity, even with low specificity (Dias & Brener, 1984; Schimuñis, 1985). In order to have this, it is recomended to lower the cut off titer of the techniques employed, and to use at least two, preferentially three, serological techniques. If, by chance, a donor have its blood rejected, this serological diagnosis may be confirmed thereafter. The important issue in this situation is to not use this blood.

c) Serodiagnosis in epidemiological studies – Again, a different approach should be applied in this situation. A high sensitivity is required, since a low prevalence population is expected to be found. But, in order to be feasible, a quick method of collection of samples should be used, as collection in filter papers, to allow transportation from the field to the Lab. The

employed techniques should be quick, easy to execute and cheap. It is preferable to employ at least two different techniques (Zicker et al., 1989).

d) Follow up of treated patients – This is the more difficult situation because we need the highest specificity, high sensitivity and the use of as many techniques as possible. It may require years of follow up, and very often, a non defined situation is obtained (with oscilating titers) (Krettli et al., 1982) when some of the techniques gave positive results, other negative and other borderline (Rassi, 1982).

PROBLEMS WITH EXISTENT TESTS AND REAGENTS

Even if results of serological tests for diagnosis of American trypanosomiasis are quite clear in the majority of cases (provided that at least two tests are employed), some situations are still unsolved with current techniques available.

A typical situation occurs when studying areas in which *T. rangeli* and *T. cruzi* infections exist at the same time. There are few studies that compare serology on this situation, when the use of purified antigens or other procedures have been recomended (Hudson et al., 1988).

Another non-solved situation occurs in areas where *T. cruzi* and any one of the leishmanias of the New World are present. Cross reactions, mainly at lower titers are often found.

Perhaps one of the areas in which there is an urgent need for new tools, is in the follow up of patients submitted to chemotherapy. Doubtfull reactions are the rule in those treated mainly in the chronic phase of the infection (Rassi, 1982). The hope that lytic assays (Krettli et al., 1982) would solve these problems, did not correspond with results obtained (Luquetti et al., 1987).

An old description of the positive xenodiagnosis patients with negative serology (Freitas, 1947), has been described in some opportunities (Luquetti, 1987, Brenière et al., 1989), and even if it is not frequent, it deserves confirmation with more accurate techniques, not currently available.

Finally, the description of spontaneous cure after a long follow up of patients in the acute phase (Zeledon et al., 1988), in the absence of treatment, and also the finding of 10% negative serology cases with megaesophagus from endemic areas (Luquetti, 1987) highlight the theory of spontaneous cure as possible. These facts should be proved with more sensitive techniques and antigens, as we way expect from recombinant proteins and purified antigens.

GOALS OF THIS STUDY

With the description by several Labs in different countries of several purified antigens and synthetic or recombinant proteins for the serological diagnosis of Chagas' disease, the need to evaluate the potentiality of each one employing standardized serum samples, has emerged.

The requirements of sera to be assayed would be to have been extensively tested by different conventional serological techniques in a reference Lab. and, perhaps more important, to know from each serum, the necessary epidemiological and clinical background of patients from whom these sera had been obtained. Secondary, a reasonable amount of serum from each patient would be needed, in order to distribute the panel among a given number of participant labs. Another requirement for the samples would be to have been preserved in suitable low temperatures, under controlled conditions.

REFERENCE LABORATORY

The Laboratory of Chagas' disease was created in 1976, by a collaboration project between the Department of Clinical Medicine, Faculty of Medicine, Federal University of Goiás (Dr J. M. Rezende and A. Rassi) and the Laboratory of Parasitic Diseases, NIH, USA (Dr F. A. Neva). Since then, well studied patients with the acute and chronic phase of Chagas' disease have been referred and more than 2,000 sera have been collected, aliquoted and stored for different purposes in several freezers and liquid nitrogen containers, under controlled condition. Serology with the standardized conventional techniques has been performed on these and on other sera and eluates from field studies (Zicker et al., 1989) gaining considerable expertise on serological techniques.

TABLE I

Epidemiological, clinical and laboratorial characteristics of Chagas' disease patients included in the study

Iden sera GOWH	Age	Sex	Epidemiology Natur State	micro	Pre-val	Clinical Main compl.	Evo-lut.	Exames performed EKG	X-ray Esoph	Colon	Laboratory Paras. xeno	Sero-logy
02	38	M	GO	354s	26%	disph	01y	nor	MII	YES	POS	POS
03	31	F	GO	350s	18%	disph	10y	nor	MIII	ND	ND	pos
05	05	M	BA	132s	8%	disph	01y	ABN	MI	nor	neg	pos
06	25	F	BA	132s	6%	disph	15y	nor	MIV	YES	POS	POS
08	52	M	MG	171o	3%	disph	22y	nor	MIV	YES	ND	POS
09	49	M	GO	360s	4%	disph	35y	ABN	MIV	nor	ND	POS
10	51	M	GO	358s	3%	disph	25y	ABN	MIII	YES	ND	POS
13	60	M	GO	358s	17%	disph	10y	nor	MIII	nor	ND	pos
14	35	M	BA	136o	4%	disph	20y	nor	MII	nor	ND	POS
15	50	F	GO	359o	11%	disph	01y	ABN	MI	nor	ND	pos
16	27	F	GO	354s	10%	obst	08y	nor	MII	SUR	neg	POS
17	32	M	GO	359s	11%	disph	03y	nor	MIV	YES	POS	POS
20	39	M	GO	354o	14%	disph	18y	ABN	MII	nor	POS	pos
22	31	F	GO	354s	9%	disph	02y	nor	MII	nor	ND	POS
23	54	M	GO	358o	5%	obst	10y	nor	MIII	SUR	ND	pos
24	34	F	GO	354s	13%	disph	20y	nor	MII	YES	neg	POS
25	13	M	BA	132s	6%	disph	07y	nor	MII	YES	neg	POS
26	14	M	BA	132s	6%	disph	05y	nor	MII	nor	POS	pos
27	31	F	BA	132s	1%	disph	05y	nor	MII	nor	neg	pos
28	49	F	GO	353s	2%	disph	02y	nor	MI	nor	neg	pos
30	43	M	GO	360s	4%	obst	03y	ABN	norm	YES	ND	pos
32	44	F	GO	359o	11%	obst	03y	nor	MI	SUR	ND	POS
40	35	M	GO	354s	11%	no	–	ABN	norm	nor	neg	pos
41	28	F	GO	358s	10%	(acPh)	23y	nor	norm	nor	POS	POS
42	58	M	GO	350s	13%	disph	10y	nor	MIV	nor	ND	POS
45	58	M	MG	172o	20%	disph	18y	ABN	MIII	nor	POS	POS
47	49	F	MG	179o	2%	disph	03y	nor	MIII	YES	neg	pos
48	68	M	GO	358o	5%	disph	12y	ABN	MIII	nor	POS	pos
49	42	F	BA	131s	1%	disph	15y	nor	MII	nor	ND	pos
Tot = 29		17M 7BA/19GO						9ABN	3norm	12YES	8POS	all

Iden: identification number GOWH: code of GOIÁS-WHO sera. Natur: place of probable contamination; micro: microregion. Preval: prevalence of positive serology cases in the region – s: single microregion; o: more than one residence. Main compl.: main complain of patiente. disph: disphagia; obst: obstipation; (acPh): documented acute phase 23 years before. Evolut.: clinical evolution of symptoms – y: years. EKG: eletrocardiogram – nor: normal; ABN: abnormal. Esoph: barium swallow – MI to MIV: degrees of megaesophagus. Colon: barium enema – YES: megacolon; ND: not done; SUR: surgery. Paras.: parasitological exams; xeno: xenodiagnosis – POS: positive; neg.: negative.

SELECTION OF PANEL OF SERA FOR THE STUDY

The first issue was to establish the minimum number of sera necessary to be included in the study. It was established that a panel of a total of 50 sera, including Chagas' disease patients, normal controls and patients with other diseases such as calazar, tegumentary leishmaniasis, and *T. rangeli* infection, would be enough, at least in a preliminary trial.

A balanced number of positive and negative samples was suggested, since it would be important to establish not only sensitivity but also specificity of the different assays.

A selection of sera from Chagas' disease patients was done by splitting them in two groups: one with high antibody titers, with the aim of having a group of easy diagnosis by any method used and other, with low (but diagnostic) titers, which would be expected to be classified as borderline by some techniques. As it would be quite difficult to have a large number of sera with these characteristics for all the four techniques employed routinely in

TABLE II

Normal individuals selected for the study

Ident. No. ser.	Age	Sex	Born	Preva-lence	Sero-logy	Remarks
04	22	M	urban	–	Neg	lab. staff
07	46	M	urban	–	Neg	lab. staff
12	33	F	354s	16.2%	Neg	obstipation, 10 days[a]
18	27	F	358s	4.8%	Neg	goitre
19	37	F	354s	16.2%	Neg	varices (leg)
21	49	F	180o	30.0%	Neg	goitre
31	33	M	357s	1.8%	Neg	no complain
33	25	M	urban	–	Neg	lab. staff
37	21	M	350o	6.8%	Neg	gastric ulcer
38	47	F	353o	5.1%	Neg	goitre
43	43	M	132o	0.6%	Neg	inguinal hernia
46	52	M	353s	1.8%	Neg	high blood pressure
Tot = 12		5F/7M			all	several

a: normal barium swallow and barium enema.
– normals were from same regions as some of the Chagas' disease patients selected for this study (see Table I).

TABLE III

Cases with muco-cutaneous and visceral leishmaniasis epidemiological, clinical and laboratorial data

No. ser.	Initials	Age	Sex	State	Clinical aspects	i/d test	Parasit. exames	Serol. Chagas
11	JVV	29	M	GO	skin	POS	path. specimen	Neg
34	GBS	16	F	BA	skin	POS	culture (L. b. b)	Neg
35	PFP	32	M	RO	skin	–	culture (L. b. b)	Neg
01	NPS	01	F	BA	viscer.	–	sternal biopsy	Neg
29	FAS	03	M	BA	viscer.	–	sternal biopsy	Neg
36	LMS	18	M	BA	viscer.	–	culture (L. ch.)	Neg
39	MV	18	M	BA	viscer.	–	culture (L. ch.)	Neg
44	DMCS	07	F	BA	viscer.	–	culture (L. ch.)	Neg
50	JP	30	M	BA	viscer.	–	culture (L. ch.)	Neg
09 cases			3F					all

i/d test: Montenegro's reaction.
culture: L.b.b.: *Leishmania braziliensis braziliensis.*
L. ch: *Leishmania (donovani) chagasi)*

our Lab. (IHA*, IIF#, DA2ME*AND ELISA*), two of the commonly employed techniques were selected: IHA and IIF.

Titers were considered "high" for IHA tests when positive at $=>$ 1/128 dilution and for IIF when equal or higher than 1/160 dilution. Lower titers were defined for IHA as 1/16-1/64 and for IIF as 1/40-1/80. Other serological reactions (ELISA and DA2ME) were positive, either in high or low titers in all samples. More than, 1,000 sera were searched, and we ended with approximately 150 sera which would fullfill those requirements.

The serum samples were also checked in relation to the amount of serum available, and complete medical history in records. Results of an EKG as well as barium swallow X-ray were mandatory. Preference was given to patients that have been probably contaminated

(*IHA and DA2ME kits and peroxidase conjugated with anti-human IgG from Imunoserum Ltda, São Paulo (R), and # fluorescein conjugated anti-human IgG from Biolab (R), São Paulo, Brazil).

TABLE IV

Serological results obtained by the reference Lab. and by the participating Labs on sera of patients with Chagas' disease

Iden sera GOWH	Reference Lab IHA	IIF titers	AD2ME	ELISA cut-off	Ab	Multicentric Network of Labs. 1	2	3	4	5	6	7	8	9	con gr.
02	4096	320	>64	1.5	H	P	P	N	P	N	P	P	P	P	7P
03	32	80	>64	1.5	l	P	P	N	P	N	P	P	P	P	7P
05	32	80	>64	1.6	l	P	P	N	P	N	P	P	P	P	7P
06	512	160	>64	2.5	H	P	P	N	P	N	P	P	P	P	8P
08	4096	160	>64	2.0	H	P	P	N	P	N	P	P	P	P	7P
09	512	640	>64	2.9	H	P	P	P	P	N	P	P	P	P	8P
10	256	640	>64	2.4	H	P	P	P	P	N	P	P	P	P	8P
13	32	80	64	1.8	l	P	P	P	P	N	P	P	P	P	8P
14	256	160	64	2.0	H	B[a]	N	N	N	N	P	P	P	P	5P[b]
15	64	80	>64	1.7	l	P	N	N	P	N	P	P	P	P	6P[b]
16	256	640	>64	2.3	H	P	P	N	N	N	P	P	P	P	6P[b]
17	4096	320	256	2.5	H	P	P	P	P	N	P	P	P	P	8P
20	64	80	>64	1.9	l	P	P	N	P	N	P	P	P	P	7P
22	512	160	>64	2.9	H	P	P	N	P	P	P	P	P	P	8P
23	32	80	64	2.9	l	P	P	N	P	P	P	P	P	P	8P
24	512	320	>64	2.9	H	P	P	N	P	P	P	P	P	P	8P
25	256	640	>64	2.5	H	P	P	N	P	N	N	P	P	P	7P
26	16	80	>64	2.1	l	B[a]	N	N	P	N	P	P	P	P	6P[b]
27	32	80	>64	1.8	l	P	P	N	P	N	P	P	P	P	7P
28	32	80	32	1.7	l	P	P	P	P	N	P	P	N	P	7P
30	32	80	>64	1.2	l	B[a]	P	N	B[a]	N	P	P	P	P	7P
32	128	160	>64	2.7	H	P	P	N	P	P	P	P	P	P	8P
40	32	80	16	1.4	l	P	P	N	P	N	P	P	P	P	7P
41	1024	640	128	2.0	H	P	N	P	P	N	P	P	P	P	7P
42	512	640	128	1.6	H	P	P	N	P	N	P	P	P	P	7P
45	512	640	64	2.1	H	P	P	N	N	N	P	P	P	P	6P[b]
47	32	40	64	1.5	l	P	N	P	P	N	P	P	P	P	7P
48	16	80	64	1.2	l	P	N	N	P	N	P	P	P	P	6P[b]
49	32	80	>64	1.9	l	B[a]	P	N	P	N	P	P	P	P	7P

Tot = 29

a: borderline results, as selected by participant.
Ab: antibody titers – l: low titers by IHA and IIF. H: high titers by same techniques (see text).
P: positive serum as selected by participant. N: negative congr: number of Labs that selected a given serum as positive, in congruence with results obtained by Reference Lab.
b: those sera that showed lower congruence between Labs and Reference Lab.

in a single geographical region (those who have lived always in the same place until eventually moved to large cities). Care was taken to select those cases that never have been submitted to specific chemotherapy for Chagas' disease.

Conditions above were fullfilled by approximately 80 sera. After localizing and defreezing adequate volumes, serological reactions with IHA and IIF were repeated to confirm high or low titer. Some sera were withdrawn because the titer obtained was not the expected as by previous testing, either for one or both techniques.

An aliquot of all these sera was tested for HIV and HBs infection and only included if negative for both tests. In this case they were mixed with equal amounts of glycerine, aliquoted, and tested again for their IHA/IIF titers.

Twenty nine sera were selected (Table I), a random order number between 1 and 50 was assigned to each sample, which were then labelled and stored until distributed.

Normal controls: three sera from laboratory workers which have been bled in several opportunities, always with negative results, were included. Nine sera from non infected individuals, from endemic areas, which have been

TABLE V

Serological results obtained by the reference Lab. and by the participating Labs on sera from normal individuals

Iden sera GOWH	Reference Lab IHA	IIF titers	AD2ME	ELISA cut-off	Multicentric Network of Labs. 1	2	3	4	5	6	7	8	9	con gr.
04	< 2	20	< 4	0.8	N	N	N	N	N	N	N	N	P	8N[b]
07	< 2	< 20	< 4	0.6	N	N	N	N	N	N	N	N	N	9N
12	< 2	< 20	< 4	0.5	N	N	N	N	N	N	N	N	N	9N
18	< 2	< 20	4	0.6	N	N	N	N	N	N	N	N	N	9N
19	< 2	< 20	< 4	0.5	N	N	N	N	N	N	N	N	N	9N
21	< 2	< 20	< 4	0.6	N	N	N	N	B[a]	N	N	N	N	9N
31	< 2	< 20	< 4	0.4	N	N	N	N	B[a]	N	N	N	N	9N
33	< 2	20	< 4	0.7	N	N	N	N	N	N	N	N	N	9N
37	< 2	< 20	< 4	0.5	N	N	N	N	N	N	N	N	N	9N
38	< 2	< 20	< 4	0.6	N	N	N	N	P	N	N	N	N	8N[b]
43	< 2	< 20	4	0.5	N	N	N	N	N	N	N	N	N	9N
46	< 2	< 20	4	0.6	N	N	N	N	N	N	N	N	N	9P

Tot = 12

a: borderline results, as selected by participant, were included as negative in this table.
P: positive serum as selected by participant. N: negative cong: number of Labs that selected a given serum as negative, in congruence with results obtained by Reference Lab.
b: those sera that showed lower congruence between Labs and Reference Lab.

TABLE VI

Serological results obtained by the reference Lab. and by the participating Labs on sera from muco-cutaneous and visceral leishmaniasis

Iden sera GOWH	Reference Lab IHA	IIF titers	AD2ME	ELISA cut-off	Multicentric Network of Labs. 1	2	3	4	5	6	7	8	9	con gr.
11	< 2	40	< 4	0.7	N	N	N	N	N	N	N	N	P	8N
34	< 4	< 20	< 8	0.7	N	N	N	N	P	N	N	N	N	8N
35	< 2	< 20	< 8	0.6	N	N	N	N	N	N	N	N	N	9N
01	< 2	< 20	< 4	0.8	N	N	N	N	P	N	N	P	P	6N[b]
29	< 4	< 20	< 8	0.6	N	N	N	N	P	N	N	N	P	7N[b]
36	< 4	20	8	0.8	N	N	P	N	P	N	N	N	N	7N[b]
39	< 4	< 20	8	0.9	N	N	N	N	P	N	N	N	N	8N
44	< 4	< 20	< 8	0.8	N	N	B[a]	N	N	N	N	N	N	9N
50	< 4	40	< 8	0.6	N	N	N	N	N	N	N	N	P	8N

Tot = 09

a: borderline results, as selected by participant, were included as negative in this table.
P: positive serum as selected by participant. N: negative congr: number of Labs that selected a given serum as negative, in congruence with results obtained by Reference Lab.
b: those sera that showed lower congruence between Labs and Reference Lab.

extensively tested, were also included as normal controls. (Table II). Care was taken to choose some normals from endemic areas with more than 5% prevalence as reported in the National Serological Survey (1975-1981) (Camargo et al., 1984).

The last group of sera included was from leishmaniasis patients. We included two cases from our own serobank. Other two sera from patients with cutaneous leishmaniasis were from Universidade de Brasília, (kindly provided by Dr Cesar Cuba Cuba), with parasitological

diagnosis (a positive culture with *Leishmania brasiliensis brasiliensis*). Cases of calazar with proven parasitological diagnosis (amastigotes in bone marrow) and cultures with growth of *L. (donovani) chagasi* were from the Hospital Edgard Santos, (kindly provided by Dr Edgar M. Carvalho Filho) (Table III). These two categories of sera were also submitted to HIV and HBs tests, as well as repeated tests by the four conventional serological techniques available.

All samples were adequately distributed in a volume of 120 μl in a polietilene tubing (#210), heat sealed, rotulated, conditioned in corrugated hard paper (Camargo et al., 1986) and posted in simple envelopes, in three parcels, together with appropriate forms to be filled in. Instructions were given to participants in relation to test each sample by their methods and antigens, and inform, for each serum, if positive, negative or borderline; cut off levels would be fixed by each Lab. The final conclusion for a given sera should be sent for analysis by the Reference Lab. and by the Secretariat of the Steering Committee on Chagas' disease.

From 14 Labs invited all around the world, 10 replied with interest in participating in the study. From them, there was only one withdrawal, due to the short time required to process samples and send results.

Contacts were established between the network of Laboratories in order to monitorize arriving of samples. From the first parcel of 10 envelopes sent, two were lost, and from the second parcel, one envelope was lost. New samples were sent in time for those Labs, as well as extra samples of the same panel, when required. The loss of panels (10%) was attributted to the poor Postal Service in two of the countries involved. To correct this problem, further samples to these countries were sent only by Express Post, which increased the cost by 100 times (U$S 0.50 to U$S 50.00).

RESULTS

In Table IV, results from sera of chagasic patients with four conventional serological techniques are shown, together with results of each participating Lab. On Tables V and VI, results obtained with sera from normal and leishmaniasis patients are included.

It can be seen that at least 5 out of 9 Labs presented a concordant result for each one of the coded positive samples with the reference Lab. With the negative samples from normal individuals, two Labs had a false positive result. From coded negative samples of leishmaniasis patients, a concordance of results was obtained in at least 6 Labs, for each sample.

An analysis of the performance of each Lab. and each antigen is shown in a separate paper, in this issue (Moncayo & Luquetti, 1990).

At the end of the study, and after receiving all the results from the Labs involved, the codes for each serum were broken and sent to each participant.

FINAL REMARKS

– The panel of sera selected for this study showed to be appropriate, as the majority of Labs identified each sample in agreement with the reference Lab.

– The degree of collaboration between different centres was excellent, and quick communication allowed to complete all the study in less than four months between the acceptance in participating in the study until the results were received back.

– This work did show the feasibility of performing a multicentric study in a short period of time, succesfully, and we hope will encourage future studies on similar basis.

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