

## CHAGAS SEROLOGY AND ITS PROBLEMS

by

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*Summary* — We performed a comparative study (263 sera) of four serological techniques for the diagnosis of Chagas' disease (IFT, CFT, ELISA, IEP). The concordance of the four techniques was 92.8 per cent, and we obtained good quantitative correlations between IFT, CFT and ELISA techniques. Looking at the positivity or negativity of three out of four techniques in order to sort out positivity and negativity of the sera, we obtained a relative sensitivity for the techniques from 96 per cent to 100 per cent, and a relative specificity from 95.1 per cent to 100 per cent. On the other hand, we evaluated the ELISA and IEP techniques. Both techniques appeared to be valuable for Chagas' diagnosis.

150 patients were submitted to xenodiagnosis and their sera were analysed for Chaga's disease. Four of them without any previous treatment appeared to have a peculiar status with positive xenodiagnosis and negative serology. The authors suggest the systematic practice of xenodiagnosis and serodiagnosis. Besides, the development of a test for detecting circulating antigens of *T. cruzi* could be useful for the implementation of the classical serology on these patients.

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KEYWORDS : Chagas' Disease, Serological Diagnosis; ELISA; Immuno-electrophoresis.

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### Introduction

The classical serology of Chagas' disease is performed by various techniques, the most useful of which are immunofluorescence (IFT) and complement fixation (CFT) tests. In the present work, we evaluate the enzyme linked immunosorbent assay (ELISA) and immunoelectrophoresis (IEP) techniques. In the other hand, we discuss on the limits of validity of the classical serology. The first problem which has been discussed in other studies is that of the specificity. In fact, a highest specificity for Chagas' disease is required when in a same place coexist various infections likely to produce cross reactions in the Chagas' diagnosis (Brénière *et al.*, 1985). The second feature is the existence of sera from confirmed Chagas patients presenting a very low titer of antibodies that are considered negative by classical serology. We report here 4 cases out of 150 patients examined.

## Materials and Methods

Epimastigote forms of *T. cruzi* (Tehuentepec strain) were obtained from cell free culture in GLSH monophasic medium at 28 °C (Le Ray, 1975). The parasites were collected by centrifugation at 2,000 g and washed three times in Hanks-Wallace solution (Hanks & Wallace, 1949). Then the parasites were suspended in NaCl 1‰, desintegrated using an hydraulic press at 1800 PSI (X Press LKB) and centrifugated at 2,600 g for 1 hour at 4 °C. The supernatant was dialysed and lyophilized. This total *T. cruzi* antigenic extract was used for CFT and ELISA tests. As for the precipitation test in agarose (IEP) we directly used antigenic extract obtained after passing three times by the hydraulic press. For the IFT test, the epimastigotes of *T. cruzi* were fixed with glutaraldehyde according the Weller & Coons (1954) procedure.

### Human sera :

- 263 sera from adult patients living in different endemic areas of Bolivia were studied, to compare four serological techniques (IFT, CFT, ELISA, IEP);
- 68 sera were used as controls, and came from Bolivian people of the highlands (Altiplano) without Chagas' disease;
- 10 sera were from European people who never lived in endemic areas;
- 150 patients from endemic areas were investigated by the four serological techniques and the xenodiagnosis.

### Serological tests :

**IFT:** Indirect immunofluorescence test was according to Weller and Coons (1954). The fluorescent labelled antihuman immunoglobulin conjugate (Institut Pasteur, France) was used diluted 1/200. The significant dilution of sera for diagnosis of Chagas' disease was 1/40.

**CFT:** Complement fixation described by Guerreiro Machado (1913), modified by Kent and Fife (1963) was used. The significant dilution of sera for Chagas' disease was 1/2.

**ELISA:** Enzyme linked immunosorbent assay was according to Carlier *et al.* (1980). The peroxydase labelled total antihuman immunoglobulin (Institut Pasteur, France) was used at 500 ng/ml and absorbance values were measured at 405 nm. The positivity limit of the test was 0.17 (O.D.).

**IEP:** Immunoelectrophoresis was carried out according to Afchain *et al.* (1976) in 1 per cent agarose using three times concentrated sera. We considered the technique positive according to the number of precipitation lines (test always positive when at least three arcs) and intensity (test positive with 1 or 2 intense arcs).

### Xenodiagnosis :

Patients were exposed for 30 minutes to 30 *Triatoma infestans* specimens of third stage. Faeces control was carried out one, two and three months after the insect bite. This observation was performed on microscope slide by pooling faeces from three triatomines.

## Results

### Evaluation of ELISA and IEP techniques :

Initially, we made the evaluation of ELISA and IEP tests with the 263 patient sera from different endemic areas. Two types of analysis have been done :

— quantitative correlation study between IFT, CFT and ELISA, with the following results :

	r	t	p
IFT/CFT	0,23	3,41	< 0,001
CFT/ELISA	0,32	4,76	< 0,001
IFT/ELISA	0,43	9,36	< 0,001

(r = correlation coefficient; t = value of t of student; p = probability).

— study of the agreement and disagreement between the four techniques (Fig. 1). 190 and 54 sera were positive and negative respectively

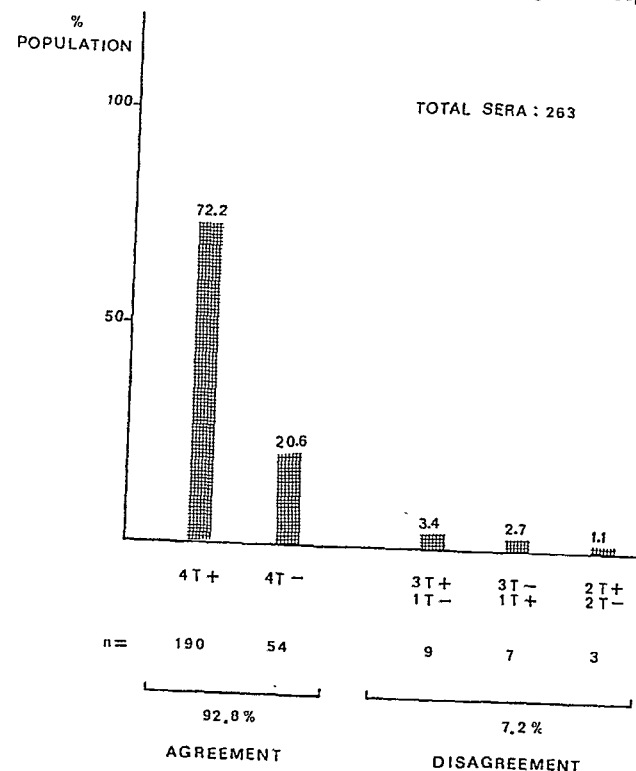


Figure 1.  
Agreement and disagreement of IFT, CFT, ELISA and IEP for Chagas' disease.  
n = number of sera; T+ = positive technique; T- = negative technique.

with the four techniques. For 7.2 per cent of sera, the four techniques were not in agreement, for 6.1 per cent three techniques out of four agreed in terms of positive or negative results, and 1.1 per cent of the sera were considered as doubtful because two techniques were positive and two were negative.

Each technique was evaluated according to the diagnosis based on the positivity or negativity of three out of four techniques (Table 1). The co-positivity of the results which means the relative sensitivity of the techniques and also the co-negativity which means the relative specificity of them showed that there were no significant differences between the studied techniques.

TABLE 1  
Evaluation of the IFT, ELISA, CFT and IEP techniques according to negativity or positivity of three out of four techniques

Tests	Agreement		Co-positivity		Co-negativity	
	No.	%	No.	%	No.	%
IFT	252	99.2	199	100.0	59	96.7
ELISA	257	98.8	199	100.0	58	95.1
CFT	257	98.8	198	99.5	59	96.7
IEP	252	96.9	191	95.0	61	100.0

#### Depression of specific antibodies to *T. cruzi* in patients with a positive xenodiagnosis:

150 patients have been submitted to xenodiagnosis and their sera were analysed for Chagas' disease. Four patients with positive xenodiagnosis (2.7 per cent) presented a peculiar immunological status with a negative serology (Table 2).

TABLE 2  
Parasitological and serological results of 150 chronic chagasic patients

	Patients		Xenodiagnostic (+)		Xenodiagnostic (-)	
	No.	%	No.	%	No.	%
Serology +	146	97.3	92	61.3	54	36
Serology -	4	2.7	4	2.7	—	—

Details of the serological status of these 4 patients are exposed in Table 3. None of these patients had received any treatment and the clinical examination was normal.

TABLE 3  
Serology and xenodiagnosis of four patients with depression of specific antibodies to *T. cruzi*

Patients	Age	Dates of analysis	IFT	ELISA	IEP	Xenodiagnosis
Patient 1	18	Nov. 1981	+	+	+	-
		Nov. 1982	-	-	-	+
Patient 2	48	May 1981	-	-	-	+
		Jan. 1983	-	-	-	-
Patient 3	30	Nov. 1982	-	-	-	+
		Dec. 1982	-	-	-	-
Patient 4	31	Apr. 1980	-	-	-	+

Patient No. 1 had a negative serology, one year after a previous positive test with a negative xenodiagnosis which became then positive. Patient No. 2 maintained a negative serology two years after the first test, while the xenodiagnosis, primarily positive, turned then negative. Patient No. 3 presented a negative serology one month after a first negative test, and has also a positive xenodiagnosis. Patient No. 4 showed also a negative serology and a positive xenodiagnosis.

#### Discussion

The comparative study of the techniques showed a good agreement between CFT, IFT, ELISA and IEP, since no significant differences could be observed between the results.

Our results are in concordance with those of Camargo *et al.* (1977) who obtained a relative sensitivity of 99.9 per cent for IFT and 99.2 per cent for CFT; Fuchs *et al.* (1980) obtained sensitivity of 98.5 per cent with ELISA, 95.1 per cent with IFT and 73.1 per cent with CFT. Spencer *et al.* (1980) noted 87.4 per cent agreement between ELISA, IFT and CFT. Moreover, the ELISA and IEP techniques can be useful for Chagas' serodiagnosis. The ELISA technique uses small quantities of antigen, it is of rapid execution and a great number of samples can be proceeded at the same time. The IEP technique is worthwhile for a laboratory with little equipment. On the other hand, this technique shows a high specificity and the visualisation of arc 5 (Afchain *et al.*, 1979) can be helpful in some doubtful cases.

Concerning the cases with depression of antibodies to *T. cruzi*, it would be suggested to make the serodiagnosis and xenodiagnosis at the same time to avoid losing the patients. On the other hand, a systematic practice of xenodiagnosis and serodiagnosis in an endemic area could allow a real evaluation of the frequency of these patients. Out of the four patients presenting no humoral antibody response to *T. cruzi*, two were asymptomatic and the others presented a cardiac pathology. The patients with no signs of immune response could experience in the future a worsening of illness. Moreover, the xenodiagnosis detects only 50 per cent to 60 per cent of Chagas' patients in the chronic phase, so we can hope that the development of new tests for the dosification of circulating antigens would help the diagnosis of these patients.

#### La sérologie de la maladie de Chagas et ses problèmes.

**Résumé** — Nous avons effectué une étude comparative de quatre techniques sérologiques (IFT, CFT, ELISA, IEP), à partir de 263 sérums de malades suspects de la maladie de Chagas. Une concordance de 92,8 p. cent a été obtenue entre les techniques, ainsi que de bonnes corrélations quantitatives entre les techniques d'IFT, CFT et ELISA. Prenant comme critère la positivité ou la négativité de 3 techniques sérologiques sur 4, afin de classer les sérums en positifs ou négatifs, nous avons obtenu une sensibilité relative des techniques de 96 p. cent à 100 p. cent, et une spécificité relative allant de 95,1 p. cent à 100 p. cent. De plus, ce travail nous a permis d'évaluer les techniques d'ELISA et d'IEP, qui s'avèrent utiles pour le diagnostic de la maladie de Chagas.

Le xénodagnostic fut pratiqué sur 150 patients dont les sérums étaient analysés pour la maladie de Chagas. Quatre de ces patients (aucun n'ayant reçu de traitement préalable) présentaient un statut particulier, avec un xénodagnostic positif et une sérologie négative. Les auteurs suggèrent la pratique systématique du xénodagnostic associée à celle du sérodiagnostic. Outre la sérologie classique, la mise au point d'un test permettant de doser des antigènes circulants dans les sérums pourrait permettre d'améliorer le diagnostic de ces patients.

## La serología de la enfermedad de Chagas y sus problemas.

**Resumen** — En 263 sueros hemos efectuado un estudio comparativo de cuatro técnicas serológicas para el diagnóstico de la enfermedad de Chagas (IFT, CFT, ELISA, IEP). Las cuatro técnicas estaban en concordancia en 92,8 por ciento. Se ha obtenido, por otra parte, buenas correlaciones cuantitativas entre las técnicas de IFT, CFT y ELISA. Considerando tres técnicas positivas o negativas sobre cuatro para clasificar los sueros en positivos o negativos, la sensibilidad relativa de las técnicas varía entre 96 por ciento y 100 por ciento. La especificidad relativa varía entre 95,1 por ciento y 100 por ciento. Este trabajo permitió además evaluar las técnicas de ELISA y IEP. Estas 2 técnicas resultaron válidas para el serodiagnóstico de la enfermedad de Chagas.

En 150 pacientes se practicó el xenodiagnóstico y los sueros fueron analizados para la enfermedad de Chagas. Cuatro de estos pacientes de los cuales ninguno había recibido tratamiento presentaron un estado particular con xenodiagnóstico positivo y serología negativa. Los autores sugieren se practique sistemáticamente el xenodiagnóstico y serodiagnóstico. Además de la serología clásica, el desarrollo de un test para detectar antígenos circulantes de *T. cruzi* en los sueros, permitiría un mejor diagnóstico de estos pacientes.

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