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AUTOMATED COMPLEMENT FIXATION TEST FOR THE DETECTION OF ANTI-TRYPANOSOMA CRUZI ANTIBODIES

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SUMMARY

An automated method to detect **Trypanosoma cruzi** antibodies is described, using complement fixation with an adequate automated equipment. Fifty five serum samples from patients where **Trypanosoma cruzi** antibodies were detected by Almeida's technique for complement fixation and by Indirect Immunofluorescence test, were studied by this method. The conclusion is that this automated technique can be perfectly adapted to serology services in blood banks that aim to eliminate eventual diseases transmitted by blood donors.

INTRODUCTION

Seeking the utilisation of automated equipments that have been designed for increasing rapidity and decreasing costs of the laboratorial tests, we made this work in order to standardize a serological evaluation technique important in our environment.

Several techniques are described and used in the detection and the research of the content of anti-**Trypanosoma cruzi** antibodies [3,4,5,6,8]. These techniques present rather satisfactory results that are specific, sensitive and relatively simple yet laking on rapidity which is very important when handling a large number of samples, as is the case of the serology department of blood banks that aim to exclude eventual diseases transmitted by blood donors.

We standardized, in a Technicon automated equipment, the automation in a complement fixation for detection of the anti-**Trypanosoma cruzi** antibodies, according to a method previously described [7].

In order to compare this technique with the manual complement fixation test and indirect immunofluorescence test, 55 patients that showed anti-**Trypanosoma cruzi** antibodies had their serum samples analysed. We also analysed serum samples from 10 normal individuals.

MATERIAL AND METHODS

1) **Sera** — For the different reactions we utilised 55 serum samples of individuals that showed anti-**Trypanosoma cruzi** antibodies and 10 sera samples of individuals from non endemic areas. The serum samples were collected aseptically and kept under -20°C until the moment of its use.

2) **Indirect Immunofluorescence test** (IIT) — It was performed according to the classic technique of indirect immunofluorescence, described by CAMARGO [5].

3) **Manual Complement Fixation Test** (mCFT) — It was performed according to the Almeida's technique [1]. We will not describe the technique since it is minutely reported in the original work.

4) **Automated Complement Fixation Test** (aCFT) — It was performed in the Technicon

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Autoanalyser system [9], designed for serological reactions, using a previously described technique [7]. In brief, this automated test uses a continous flow principle where all reagents are mixed automactically and the results are showed on a recorder paper after a colorimeter passage. The technique performed is correspondent to the recommended by the "Laboratory Branch Complement Fixation" [2], which are shown in Fig. 1.

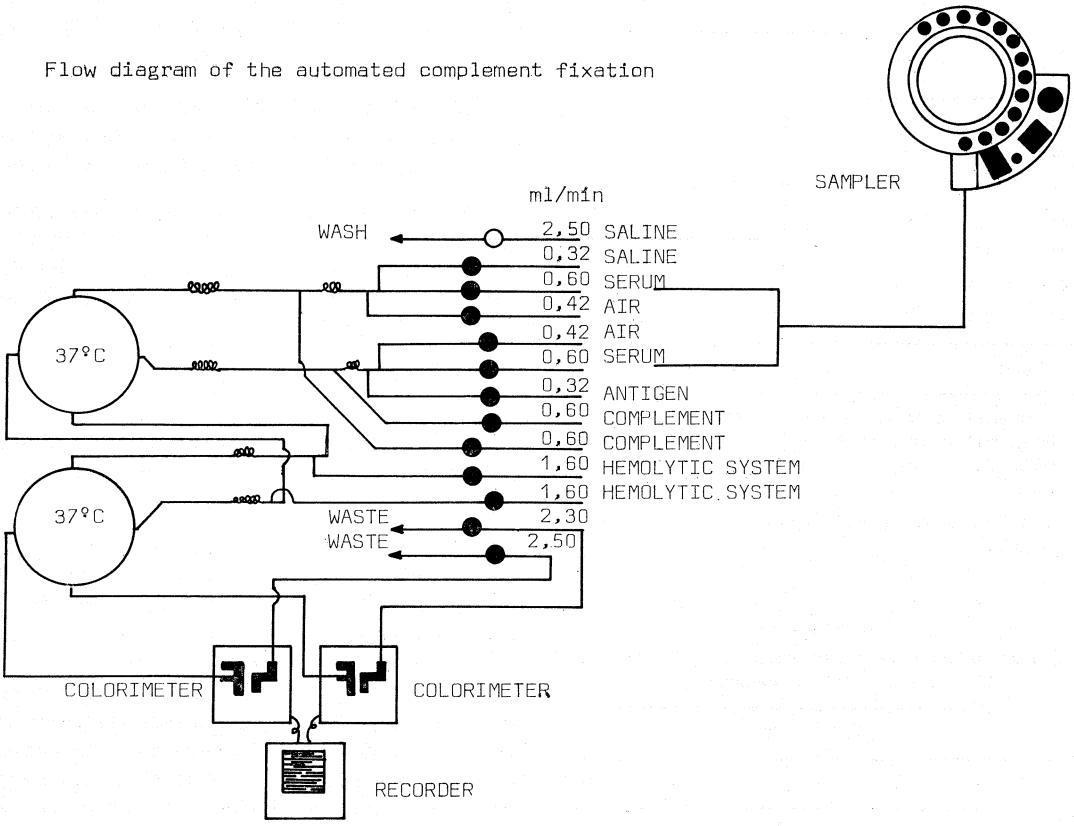

Fig. 1

5) **Reagents** — The reagents employed in this reaction, such as methylic antigen, complement, hemolysin, erythrocytes and total anti-gammaglobulin conjugate for immunofluorescence, were acquired from usual suppliers.

RESULTS

We performed the complement fixation test, in a total of 55 serum samples from patients who showed anti-**Trypanosoma cruzi** antibodies by the immunofluorescence and manual complement fixation tests, and in 10 serum samples from normal individuals.

The comparison between the results obtained by these technique are presented in the Tables I and II.

The 10 serum samples, from normal individuals, showed coherent results, that is, negative by these two techniques studied.

DISCUSSION

The available techniques for the detection of anti-**Trypanosoma cruzi** antibodies, although sensitive and precise, are not very rapid and therefore not very suitable for blood banks serology laboratories which have high work load.

We believe that this automated equipment fullfills the required needs for rapidity of execution. We also believe that the standardization of this method, with commercially available reagents simplifies the execution and

T A B L E I

Comparison between the results obtained by the aCFT and mCFT, in a total of 55 examined sera

			aCFT — Titers					
			—	2	4	8	16	Total
mCFT Titers(')								
1200	1/4	1/2(")	1	2	4	2		9
1600	1/16	1/4				2	3	5
2400	1/8	1/4		2	5	2	3	12
3200	1/32	1/8				1	4	5
4800	1/16	1/8				2	11	13
9600	1/32	1/16			2		6	8
19200	1/64	1/32					3	3
Total			1	4	11	9	30	55

(') Evaluated in terms of serum necessary for 50% of lysis, and determined by the value of the angle of inclination of immune complex-complement regression line.

(") Dilutions obtained with 3μ and 6μ of complement (CH50).

T A B L E II

Comparison between the results obtained by the aCFT and IIT, in a total of 55 examined sera

	aCFT — Titers					
	—	2	4	8	16	Total
IIT						
80	1	4	5	2	2	14
160			3	6	13	22
320				1	6	7
640			3		6	9
1280					3	3
Total	1	4	11	9	30	55

gives better reproductibility of results among different laboratories that use the same method.

From the results we conclude that the IIS is more sensitive than the other techniques, which is in accordance with current literature [3, 4, 6, 8]. The two different methods of complement fixation test have about the same sensitivity, although the titers are expressed in different ways. Besides the rapidity of execution (nearly 40 tests per hour) of this automated complement fixation method, it is possible to obtain a relative quantitative result; or, we can give the quantitative result obtained without previous serum dilution, only with titers 2, 4, 8 and $\geqslant 16^7$, being an economical and easy way to work with many samples.

We believe that this method may be employed for the detection of antibodies originated by the other bacterial, parasitic or viral agents.

This would greatly help the accomplishment of a series of researches and evaluations that are not being made actually due to the difficulty in performing the reaction in a large number of samples.

RESUMO

Reação de fixação do complemento automatizada para detecção de anticorpos anti-Trypanosoma cruzi

Um método automatizado para a detecção de anticorpos anti-**Trypanosoma cruzi** é descrito. O método utiliza a técnica de fixação do complemento em equipamento adequado para a realização de testes automatizados. Foram estudados por este método, 55 amostras de soro de pacientes onde esses anticorpos foram detectados pelas técnicas de complemento descrita por ALMEIDA [1], e teste de imunofluorescência indireta. Concluímos ser esta técnica automatizada perfeitamente adaptável a serviços de sorologia de Bancos de Sangue que visam eliminar eventuais doenças transmitidas por doadores de sangue.

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