Rev. Inst. Med. trop. São Paulo
8(6):255-258, novembro-dezembro, 1966

UDC 616.937.3
595.75

THE USE OF FILTER PAPER BLOOD SMEARS IN A PRACTICAL FLUORESCENT TEST FOR AMERICAN TRYPANOSOMIASIS SERODIAGNOSIS

Sebastião Loureiro de SOUZA (1) and Mário E. CAMARGO (2)

SUMMARY

Eluates of blood collected on filter paper were used in the fluorescent test for American trypanosomiasis serodiagnosis. Results obtained have shown as close an agreement with complement fixation tests as when sera is employed. Maintaining dried blood smears at room temperature for as long as 30 days did not affect reactivity of samples.

This makes even more practical the use of the fluorescent technique specially when applied to large populational surveys.

INTRODUCTION

The prevalence of *Trypanosoma cruzi* infection is known to be high in many South-American regions. However, its real incidence can only be determined through extensive serological surveys. The usefulness of practical tests for serodiagnosis is obvious.

Fluorescent antibody techniques represent a new approach to the problem since they are simple to perform and have considerable sensitivity. Fluorescent tests have already been successfully applied to the serodiagnosis of several parasitic diseases[4, 6, 9 13] including a few caused by haemoflagellates[14, 15].

FIFE & MUSCHEL[8] were the first to employ a fluorescent test for the diagnosis of Chagas' disease. They used *T. cruzi* cultural forms and reactions were carried out in test-tubes. Later, SADUN et al.[12] performed reactions on microscope slides with *T. cruzi* blood forms as antigen. BIAGI et al.[2] also described a slide test, the antigen consisting in *T. cruzi* tissue forms in infected rat myocardium sections.

The use of preserved *T. cruzi* cultural forms in a slide test was described by one of us[3]. As many sera were tested on each slide, numerous reactions could be run at the same time. Also, the stability of the antigen, mainly when freeze-dried, made the fluorescent test very practical for routine use.

As a further advantage, fluorescent tests may be carried out with minute amounts of sera or even of eluates of blood collected on filter paper, as described by ANDERSON et al.[1]. Also, in *T. rhodesiense* infection, SADUN et al.[12] observed close agreement of results between fluorescent tests made with sera and with blood smear eluates.

It has been our purpose to evaluate the reliability of the *T. cruzi* fluorescent test with blood smear eluates as compared to the complement fixation test made with sera. Eluates obtained within various periods after the blood was collected were also compared.

MATERIAL AND METHODS

Blood was obtained from 192 patients through venipuncture and finger prick.

(1) CAPES fellow at the Departament of Hygiene and Preventive Medicine, Faculdade de Medicina de Ribeirão Prêto, Ribeirão Prêto, Estado de São Paulo, Brasil. (2) Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil.

Finger blood drops were spread on circular areas marked on Whatman no. 1 filter paper. After drying for a few minutes at room temperature, the smears so obtained were kept at room temperature until used, the filter paper sheets separated from each other by unabsorbent paper. For elution, a small 4 sq. cm disc was cut from each smear and soaked in 0.25 ml buffered saline solution (NaCl 0.15 M; phosphates 0.01 M; pH 7.2) in a 12 x 75 test tube. In order to wet the filter paper discs thoroughly these tubes were maintained almost horizontally for about 30 minutes and then returned to the vertical position. Brown eluates collected in the bottom of the tubes while the whitened discs adhered to the walls, as shown in Fig. 1. As about 0.08 ml of the blood are absorbed in 4 sq. cm areas of the filter paper employed, eluates obtained corresponded to about a 1/5 serum dilution. When collecting blood, several smears were made for each patient and later different eluates were obtained and tested at various subsequent intervals. For 160 patients, this was done three times; on the 15th, 16th and 30th days after preparing blood smears. For the remaining 32 patients five eluates were obtained respectively on the 2nd, 5th, 8th, 15th and 30th days.

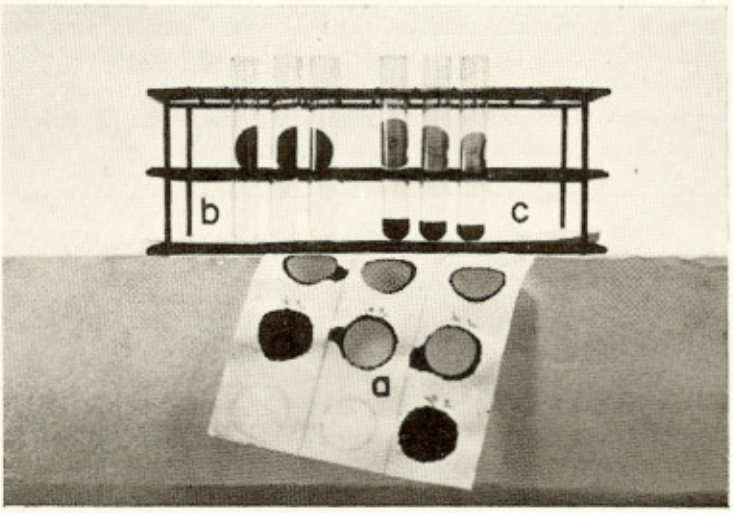

Fig. 1 — Blood smears and eluates. Discs cut from filter-paper smears (a) are shown before (b) and after (c) soaking in saline. Eluates collect in the bottom and whitened discs adhere to the walls of the test-tubes.

Fluorescent test — This was performed as previously described[3]. *Trypanosoma cruzi* cultural forms washed and fixed for 24 hours in 1 per cent formalin were freeze-dried in 6 per cent dextran. Upon reconstitution the resulting suspension was pipetted on small areas marked on microscope slides, as previously indicated[5]. Fixation was obtained by drying. An indirect or delayed test was run by incubating first eluates and then antihuman globulin conjugate on such areas, at 37°C for periods of one hour each. Evans' blue was used as a counterstain. Twenty tests were performed on each slide. Reading the tests and reporting results were carried out as previously described[3]. Care was taken to have the tests performed by people unaware of any results already obtained for the same cases.

Complement fixation tests — Inactivated sera were tested by a 50 per cent haemolysis quantitative complement fixation technique, as described by Pedreira de Freitas[11].

RESULTS

Successive tests employing eluates obtained at various intervals after blood collection have shown a close agreement of results. A decrease of reactivity was not observed even when blood was eluted 30 days after collecting. In the group of 32 samples tested five times, results were consistent in 30, of which 20 positive and 10 negative. In the remaining two cases results were divergent. In the group of 160 cases tested three times, 99 were reactive and 59 non-reactive in all tests and only 2 cases have shown divergent results. However, in three of the total four divergent cases there were only isolated positive results followed by several negative tests. It was later found that this may have been caused by insufficient fixation of the antigen to the slides and occasional adherence of stained trypanosomes released from other antigenic areas during the washing of the slide. This difficulty was overcome in subsequent tests.

In the fourth case with divergent results, only the test made on the 30th day of collection was negative, four previous tests resulting in weak (1+) positive results. It is to be remarked that weak fluorescent results were rarely seen, fluorescence being intense (3 to 4 +) for most reactive cases.

In comparing fluorescent and complement fixation tests, a close agreement was also observed. All the 69 cases constantly non-reactive in fluorescent tests were also non-reactive in the complement fixation test. From the 119 constantly reactive cases in fluorescent tests, 117 were reactive in the complement fixation test and only 2 were non-reactive. These two came from an endemic area, one of them showing no clinical evidences of Chagas' disease but the other presenting clinical signs of megacolon, the digestive form of the disease[7, 10]. Two relatives of this patient (the father and one brother) have Chagas' disease. The four cases with divergent results in the fluorescent tests were non-reactive in the complement fixation test. Table I summarizes results obtained.

TABLE I

Cases distributed according to results in successive fluorescent tests and in complement fixation test for Chagas' disease

Fluorescent tests	Complement fixation		Total
	Reactive	Non-reactive	
Reactive	117	2	119
Non-reactive	0	69	69
Divergent results	0	4	4
TOTAL	117	75	192

No correlation was found between titers in complement fixation tests, which covered a wide range, and the observed intensity of fluorescence.

DISCUSSION

We have found the use of eluates of blood, collected and dried on filter paper, a very practical and reliable technique in the fluorescent test for American trypanosomiasis serodiagnosis. In a previous paper[3] we have shown a close agreement between fluorescent and complement fixation tests when sera were employed. Results here reported indicate as close an agreement when eluates of blood collected on filter-paper were used in the fluorescent test.

Many advantages derive from this method since unskilled personnel can be used for blood collecting; smears can be sent to the laboratory by ordinary mail and even when kept at room temperature (about 20°C) for as long as 30 days, their reactivity remains unimpaired. On the other hand, since the antigen can be easily stored and since numerous tests may be run in a short time, the laboratory work is much facilitated. In this study, as many as 640 fluorescent tests were performed and 80 to 120 could be easily carried out in a single batch by only one technician. As haemolysis or anticomplementarity of sera do not affect fluorescent test results and as only minute amounts of diluted sera are necessary, blood smear eluates are perfectly suitable for fluorescent tests.

All these advantages make the test here described very useful for large scale surveys of Chagas' disease.

RESUMO

Emprêgo de sangue, colhido em papel de filtro, em reações de imunofluorescência para o diagnóstico sorológico da tripanosomíase americana

Na reação de imunofluorescência para o diagnóstico sorológico da tripanosomíase americana, em lugar de sôro, foi utilizado sangue colhido em papel de filtro, eluido em solução salina tamponada. Houve concordância estreita com os resultados da reação de fixação do complemento com antígeno de *Trypanosoma cruzi*, realizada com soros dos mesmos pacientes. O sangue colhido em papel de filtro não mostrou perda de atividade quando mantido sêco por períodos de até 30 dias.

Pela técnica apresentada a reação de fluorescência torna-se muito prática e assim parece-nos razoável o seu emprêgo em amplos inquéritos sorológicos.

REFERENCES

1. ANDERSON, R. I.; SADUN, E. H. & WILLIANS, J. S. — A technique for the use of minute amounts of dried blood in fluorescent antibody tests for schistosomiasis. *Exp. Parasit.* **11**:111-116, 1961.

2. BIAGI, F.; TAY, J. & MURRAY, R. M. — La reacción de inmunofluorescencia en el diagnostico de la Enfermedad de Chagas. *Bol. Ofic. Sanit. Panamer.* **57**:234-240, 1964.

3. CAMARGO, M. E. — Fluorescent antibody test for the serodiagnosis of Chagas' disease. Technical modification employing preserved cultural forms of *Trypanosoma cruzi* in a slide test. *Rev. Inst. Med. trop. São Paulo* **8**:227-234, 1966.

4. CAMARGO, M. E. — Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. *Rev. Inst. Med. trop. São Paulo* **6**:117-118, 1964.

5. CAMARGO, M. E. — Preparation of microscopical slides to simplify immunofluorescent serological titrations. *Rev. Inst. Med. trop. São Paulo* **7**:39-40, 1965.

6. CAMARGO, M. E.; HOSHINO, S. & SILVA, L. C. da — A slide fluorescent antibody technique with adult worm antigen for the serological diagnosis of schistosomiasis *mansoni*. *Rev. Inst. Med. trop. São Paulo* **7**: 327-331, 1965.

7. CHAGAS, C. — Processos patogênicos da tripanosomíase americana. *Mem. Inst. Oswaldo Cruz* **8**:5-36, 1916.

8. FIFE Jr., E. H. & MUSCHEL, L.H. — Fluorescent antibody technique for serodiagnosis of *Trypanosoma cruzi* infections. *Proc. Soc. Exp. Biol. Med.* **101**:540-543, 1959.

9. KELEN, A. E.; AYLLON-LEINDL, L. & LABZOFFSKY, N. A. — Indirect fluorescent antibody method in serodiagnosis of toxoplasmosis. *Canad. J. Microbiol.* **8**:545-554, 1962.

10. KOEBERLE, F. — Die Chagaskrankheit: ihre Pathogenese und ihre Bedeutung als Volksseuche. *Z. Tropenmed. Parasit.* **10**:236-267, 1959.

11. PEDREIRA DE FREITAS, J. L. — Reação de fixação do complemento para diagnóstico da moléstia de Chagas pela técnica quantitativa. *Arq. Hig.* (São Paulo) **16**:55-94, 1951.

12. SADUN, E.H.; DUXBURY, R.E.; WILLIAMS, J. S. & ANDERSON, R. I. — Fluorescent antibody test for serodiagnosis of African and American trypanosomiasis in man. *J. Parasit.* **49**:385-388, 1963.

13. SADUN, E. H.; WILLIAMS, J. S. & ANDERSON, R. I. — A fluorescent antibody technique for the sero-diagnosis of schistosomiasis in humans. *Proc. Soc. Exp. Biol. Med.* **105**: 289-291, 1960.

14. SHAW, J. J. & VOLLER, A. — The detection of circulating antibody to Kala-azar by means of immunofluorescent techniques. *Trans. Roy. Soc. Trop. Med. Hyg.* **58**:349-352, 1964.

15. WILLIAMS, J. S.; DUXBURY, R. E.; ANDERSON, R. I. & SADUN, E. H. — Fluorescent antibody reactions in *Trypanosoma rhodesiense* and *T. gambiense* in experimental animals. *J. Parasit.* **49**:380-384, 1963.

Recebido para publicação em 25/5/1966.