

Long-term preservation of blood samples for diagnosis of *Trypanosoma cruzi* infection

A. C. Pérez, E. Cura, E. Subías, J. C. Lansetti, E. L. Segura

Instituto Nacional de Diagnóstico e Investigación de la Enfermedad de Chagas "Dr. Mario Fatala Chabén" (INDIECH).
Secretaría de Salud Ministerio de Salud y Acción Social, Argentina

Abstract

Feasibility and suitability for field research of a whole-blood preservation method was evaluated through the screening of anti-*Trypanosoma cruzi* antibodies in 1209 samples under different conditions. Antibody reactivity of paired samples from preserved capillary blood (CBP) and sera from venous blood (VBS) were studied by specific techniques. Over 96% concordance was found on indoor studies carried out with samples without storage or after 15 or 30 days preservation of CBP at 37°C and VBS at -20°C. Outdoor studies performed at field conditions, achieved a 92.1% concordance.

Chagas' disease is a widely distributed parasitic disease in Latin America (UNDP/WORLD by BANK/WHO/TDR, 1988) produced *Trypanosoma cruzi*. Specific immune response is expressed by the permanent presence of an ample array of antibodies which appear early after and do not suffice to control the infection nor the disease (Scott and Snary, 1982). Antibodies provide the bases for detection of chagasic infection based in the use of specific techniques.

Serological surveys in rural areas are required not only for epidemiological reasons, but to measure impact of control actions (Chuit et al., 1989) and to follow-up new cases in areas under epidemiological surveillance (Paulone et al., 1988). These studies are usually performed in tropical and subtropical regions where environmental temperature can affect sample transportation to laboratories, usually placed far from the studied areas. Thus, the use of simplified methods for sampling and preservation of samples are mandatory. Among those already intended, blood preservation in filter paper, in spite of its limitation, has been widely used for the last 20 years because of its low cost and feasibility in the field (Guimaraes et al., 1986; Marinkelle et al., 1978).

Based on the effectivity of neutral glycerine in preserving sera at 50% concentration (Camargo et al., 1986), a glycerine based, whole blood-preservation method was evaluated with respect to its capability to maintain antibody reactivity for detection of specific antibodies.

Sera from venous blood samples (VBS) and preserved capillary blood (CBP) of each studied person were processed under different conditions by indirect hemagglutination (IHA) (commercial kit kindly supplied by Polychaco SACIE Arg.). Indirect immunofluorescence (IIF) (Alvarez et al., 1968) and/or ELISA (Segura et al., 1986). CBP consisted in 50 µl of blood obtained from fingertip with a gauged capillar collected in 150 µl of preserver-buffered glycerine plus proteasas inhibitors - supplied by Biotica SA Arg and kept in hermetic polystyrene tubes arranged in 60-tubes trays. Indoor evaluation, performed at INDIECH included 465 paired samples (CBP, VBS) from unselected patients which were analyzed a) by IHA 24 hs post-sampling (97/465), b) by HAI and IIF after 15 (166/465) or 30 days (202/465) of storage, CBP at 37°C and VBS at -20°C.

At outdoor study performed in field conditions in a rural area located at General Belgrano, Province of Formosa, 1200 km far from Buenos Aires, included 744 paired samples, CBP at 4°C and VBS in 50% glycerine kept at -20°C where studied, within 30 days post-sampling by IHA and IIF and mismatched results were evaluated by ELISA. Two positive reactions were considered to indicate positive result for *T. cruzi* infection.

At indoor studies 97 paired samples without storage showed a significant positive correlation ($r = 0.895$ $p > 0.05$) with a concordance of 97.9% by HAI.

Storage of CBP at 37°, after 15 days or 30 days revealed a 97% (161/166) and 96% (194/202) concordance with respect to VBS stored during equal periods at -20°C. No statistically significant differences were observed between these two groups ($p > 0.05$) (Table 1), for IHA ($p > 0.05$), and for IIF ($p > 0.05$) within each group (Table 1). The outdoor stage with sampling and transportation at field conditions showed a concordance of 92.1% (Table 2).

The liquid blood-preserve system hereby presented resulted in a high degree of concordance between samples kept under different conditions revealing an appropriate maintenance of antibody reactivity. Taking into account the extreme conditions of field sampling a 7.9% difference achieved with the sera is acceptable for outdoor epidemiological surveys (Guimaraes et al., 1986). Preliminary studies with this methodology in Brazil showed similar results (D'hooge et al., 1986).

Accepted 31 October 1989

Trop. Med. Parasit. 41 (1990) 75-76

© Georg Thieme Verlag Stuttgart · New York

Table 1 Serological results for Chagas disease in paired samples of serum preserved at -20°C and blood-with-preserver subjected to 37°C , at 15 and 30 days

Results	Blood samples preserved					
	IHA	15 days at 37°C IFI	IHA-IFI	IHA	30 days at 37°C IFI	IHA-IFI
Positive	43	49	41	55	61	49
Negative	120	115	112	145	133	134
Discordants	3	2	5	2	8	8
No. of samples	166	166	166	202	202	202
Concordance (%)	98.2	98.8	97	99	96	96

IHA = Indirect hemagglutination = reactive equal or higher 1/32 titer

IFI = Indirect immunofluorescence = reactive equal or higher 1/32 titer

Table 2 Serological results for Chagas disease, of 744 samples from Formosa, Argentina, considering final results by indirect hemagglutination, indirect immunofluorescence and ELISA for discordants, using venous and capillary blood.

Blood withr	Serum-kept in glycerine		Total
	Positive	Negative	
Positive	219	21240	
Negative	39	465504	
Total	258	486744	

Concordance = co-positive + co-negative 684/744 (92.1%)

Positive = two positive reactions being:

Indirect hemagglutination = 1/32

Indirect immunofluorescence = 1/32

ELISA = 0.02 D.O

The advantages of this methodology includes the use of whole blood which, as in the case of filter paper, may be easily obtained from fingertip by not professionally trained personnel: the use of calibrated capillars which provides a defined volumen of samples thus, overcoming one of the limitations pointed out for the filter paper method; a preserver which ensure transportation of samples at environmental temperatures; its storage up to one month at 4°C or extreme conditions and, finally, the sample maintained in liquid state thus simplifying laboratory steps. In addition, there exists an unique tube - sampling and processing - for each sample which prevents errors in identification.

This method for sampling and preservation of antibodies in whole blood proves to be feasible, safe, and simple. It appears recommendable for epidemiological surveys to fulfill the requirements imposed by follow-up studies, detection of recent infections, congenital cases, surveillance of transmission of *T. cruzi* or when serological evaluations of the impact of actions of the Control Programmes are intended.

Acknowledgement

We wish to thank Dr. Ernesto de Titto for her help in English translation, Drs. Mirta Moreno, Ana Maria De Rissio, Sergio Sosa, and Nelly Celone who gave valuable practical assistance. This work was supported by the Secretaria de Salud Ministerio de Salud y Acción Social and by UNDP/WORLD BANK/ Special Programme for Research and Training in Tropical Diseases (TDR).

References

- Alvarez, M., J. A. Cerisola, R. W. Rohwedder: Test de inmunofluorescencia para el diagnóstico de la Enfermedad de Chagas. *Bol. Chil. Parasit.* 23 (1968) 4-9
- Camargo, M. E., E. L. Segura, I. G. Kagan, J. M. Pacheco Souza, J. da Rocha Carvalho, J. F. Yanovsky, M. C. S. Guimaraes: Three years of collaboration on the standardization of Chagas' disease serodiagnosis in the Americas: an appraisal. *Bull. Of. Pan Amer. Hlth. Org.* 20 (1986) 233-244
- Chuit, R., E. Subias, A. Pérez, C. Wisnivesky-Colli, I. Paulone, E. L. Segura: Usefulness of serology as a marker of triatomids control campaigns. *Rev. Soc. Bras. Med. trop.* (in press)
- D'hooge, H., J. C. Pinto Dias, J. F. Yanovsky, E. L. Segura, C. Silva, M. Carneiro, E. Furtado Moreira: Microtécnica simplificada para serologia de Campo em Doença de Chagas. XXII Congresso da Sociedade Brasileira de Medicina Tropical, Mina Gerais, Brazil. *Rev. Soc. Bras. Med. trop.* 19 (1986) 50
- Guimaraes, M. C. S., E. A. Castilho, B. J. Celeste, O. S. Nakahara, V. Amato Neto: Almacenamiento a largo plazo de IgG e IgM en papel filtro para su uso en encuestas seroepidemiológicas de enfermedades parasitarias. *Bol. Of. Sanit. Panam.* 100 (1986) 129-142
- Mainkelle, C. J., N. de Sanchez, M. Grogl, F. Gulil: Recomendaciones para el almacenamiento de sueros absorbidos en papel de filtro bajo condiciones rurales, para el diagnóstico de infección chagásica con la prueba de inmunofluorescencia. *Rev. Inst. Med. Trop. São Paulo.* 20 (1978) 112-114
- Paulone, I., R. Chuit, A. Pérez, C. Wisnivesky-Colli, E. L. Segura: Field research on epidemiological surveillance alternative of Chagas' disease transmission: The primary health care (PHC) strategy in rural area. *Rev. Soc. Arg. Microbiol.* 1 (1988) 103-105
- Scott, M. T., D. Snary: American trypanosomiasis (chagas's disease). In: S. Cohen, K. Warren (ed.): *Immunology of parasitic infections*. Blackwell Scientific Publications, Oxford (1982) 262-287
- Segura, E. L., J. Buá, A. Rosenstein de Campanini, E. Subias, M. Esteva, M. Moreno, A. M. Ruiz: Monoclonal antibodies against the flagellar fraction of epimastigotes of *Trypanosoma cruzi*. Complement mediated lytic activity against trypomastigotes and passive immunoprotection in mice. *Immun. Lett.* 13 (1986) 165-171
- UNDP/WORLD BANK/WHO/TDR. 9th Programme Report (1988) 89-97

Analia C. Pérez

INDECH Dr. Mario Fatale Chabèn
Av Paseo Colón 568 - CP 1063
Buenos Aires, Argentina