

## Validation of a particle gel immunoassay for *Trypanosoma cruzi* antibody detection using plasma samples collected with capillary tubes

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### Abstract

**Introduction:** The Chagas disease particle gel immunoassay (PaGIA-Chagas) is a simple, fast and practical test used for the diagnosis of the chronic Chagas disease and is based on anti-*Trypanosoma cruzi* antibody detection in serum. This study aimed to validate the PaGIA-Chagas on plasma collected with capillary tubes.

**Methodology:** Serum samples from 74 *T. cruzi*-infected and 26 non-infected individuals were tested by conventional indirect immunofluorescence and PaGIA-Chagas. Later, plasma specimens collected with capillary tubes from these same individuals were tested by PaGIA-Chagas. Results from serum samples tested by IFA and PaGIA-Chagas were considered as the reference standard to determine the accuracy parameters of the particle gel tested on plasma samples. The inter-test agreement of *T. cruzi* antibody detection by PaGIA-Chagas on serum and plasma was calculated using Kappa index.

**Results:** The PaGIA-Chagas performed on plasma collected with capillary tubes had sensitivity and specificity of 99% and 100%, respectively. The crude agreement observed with the results of the PaGIA-Chagas on plasma and serum was 99% and the Kappa index was 0.975 (CI95%: 0.782 - 1.000).

**Conclusion:** PaGIA-Chagas for anti-*T. cruzi* detection on plasma collected with capillary tubes is accurate and might be indicated to reduce fieldwork time and materials in epidemiological screening of chronic Chagas disease.

**Key words:** Chagas disease, diagnosis, particle gel immunoassay, capillary tube

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### Introduction

Indirect immunofluorescence assay (IFA), indirect haemagglutination (IHA), and enzyme-linked immunosorbent assay (ELISA) are all routinely used in laboratories for both individual diagnosis and blood donor screening of *Trypanosoma cruzi* infection. The particle gel immunoassay (PaGIA-Chagas) is a more recent method developed to diagnose chronic Chagas disease. It uses red polymer particles sensitized with three different synthetic peptides representing antigen sequences of the parasite (Ag2, TcD, and TcE), as previously described by Peralta *et al.* [1]. In the presence of sera containing specific antibodies, these particles agglutinate and the result can be read visually through Sephadex gel (Diamed, MG, Brazil) present inside a microtube inserted in a plastic card [2].

Currently, PaGIA-Chagas is widely used to diagnose chronic Chagas disease and to screen blood donors free of anti-*T. cruzi* antibodies [3]. Because it is easy to use and processes samples quickly, it can

be used on a large number of samples and is suitable for chronic Chagas disease screening in epidemiological surveys. This study aimed to validate the PaGIA-Chagas on plasma collected with capillary tubes to facilitate future epidemiological screening during field surveys.

### Methodology

The subjects who participated in this study were recruited from the Outpatient Clinic for Infectious Diseases at Julio Muller Hospital in Cuiaba MT, Brazil, from November 2005 to September 2006. Most patients were seeking medical attention for clinical or electrocardiographic suspicion of chronic Chagas disease. Healthy blood donors and patients with confirmed hepatitis B, malaria, cutaneous leishmaniasis, collagenosis, and paracoccidioidomycosis were recruited from the same hospital to compose the sample set without *T. cruzi* infection. The study was approved by the Research Ethics Committee of the Julio Muller University

**Table 1.** Results of the particle gel immunoassay performed on serum or on capillary blood-derived plasma for anti-*Trypanosoma cruzi* antibody detection.

Results from plasma samples	Results from serum samples		
	Positive	Negative	Total
Positive	73	0	73
Negative	1	26	27
Total	74	26	100

Sensitivity: 99% (92% - 100%)  
Crude agreement: 99% (97.1%-100%)

Specificity: 100% (84% - 100%)  
Kappa index: 0.975 (CI95%: 0.782-1.000)

Hospital. Subject participation was voluntary and the blood collection was performed only after participants had read and signed the consent form.

A blood sample was collected from each patient to obtain serum and to fill three heparinized capillary tubes (Perfecta, São Paulo, Brazil). All blood samples were tested by IFA and PaGIA-Chagas methods for serological confirmation of the presence of anti-*T. cruzi* antibodies. To avoid bias, serum samples were coded and processed along with other samples following routine laboratory procedures. The samples were considered positive for chronic Chagas disease only when both techniques revealed positive results, as previously recommended [4].

The PaGIA-Chagas takes place inside a microtube containing Sephadex gel, that is attached to a plastic card (ID-Card, Diamed, Lagoa Santa, Brazil). Briefly, 10 µl of serum and 50 µl of polymer (ID-Chagas Polymers, Diamed, Lagoa Santa, Brazil) were added to each microtube. The card was incubated for 5 min at room temperature and centrifuged for 10 min in a specific centrifuge (ID-Centrifuge, Diamed, Lagoa Santa, Brazil). A positive result was typically indicated by the presence of a tight red line on the top of the gel surface. A negative result was indicated by the presence of a pellet of colored particles at the bottom of the microtube following centrifugation, with no agglutinated particles present within or on top of the gel.

#### *PaGIA-Chagas on plasma collected with capillary tubes*

After collection, capillary tubes were kept in a vertical position to allow erythrocyte sedimentation and were preserved at 4°C for a maximum of 7 days. Before performing the test, the capillary tube was carefully cut at the lower plasma layer interface using a small diamond cutter. The plasma volume obtained

was proportional to the height of the plasma column (around 15 mm), which was usually enough to get the 10 µl required in PaGIA-Chagas. A small rubber bulb was fitted to the capillary tube to dispense the plasma into the card microtube. The card was incubated, centrifuged, and read in the same manner as described above for serum testing.

#### *Statistical analysis*

Results from serum samples tested by IFA and PaGIA-Chagas were considered as the reference standard, to determine the accuracy parameters of the particle gel tested on plasma samples. The inter-test agreement of *T. cruzi* antibody detection by PaGIA-Chagas on sera and plasma was calculated using the Kappa index.

#### **Results**

The two conventional serological tests performed on serum samples from 100 recruited patients showed 74 positive and 26 negative results for *T. cruzi* infection. Antibody titres ranged from 1:40 to 1:640. Among 74 samples that were positive when the IFA and PaGIA-Chagas were performed on serum, 73 (98.6%) were also positive when PaGIA-Chagas was performed on plasma from the capillary tubes. The single sample that disagreed in PaGIA-Chagas on serum and plasma had a low-titre antibody in IFA (1:40). All 26 (100%) samples that were non-reactive for *T. cruzi* antibody in the reference tests also showed no reaction in the plasma samples from capillary tubes (Table 1). Therefore, the results of PaGIA-Chagas performed on plasma collected with capillary tubes had sensitivity of 99% (CI95%: 92%-100%) and specificity of 100% (CI95%: 84%-100%). The crude agreement and Kappa index between the two PaGIA-Chagas were 99.0% (CI95%: 97% – 100%) and 0.975 (CI95%: 0.782 – 1.000), respectively.

## Discussion

In endemic areas, tests for diagnosis of chronic Chagas disease are not only useful to identify infected patients for subsequent treatment, but also to screen for antibody-positive individuals in surveys and in blood banks to exclude them from blood donation. For the last few years, PaGIA-Chagas has been widely used in the diagnosis of Chagas disease in blood banks and in clinical laboratories [5-7]. The main advantages of this method are its high sensitivity and speed to perform [2]. The original technique requires venous blood collection to obtain serum or plasma, which makes it less applicable in remote field studies.

Several studies have been conducted with blood collected and stored in capillary tubes, with the purpose of demonstrating the practicality of this resource in the performance of immunoassays for the diagnosis of infectious and haematological diseases [8-10]. For example, studies evaluating indirect haemagglutination in a malaria epidemiological survey showed that blood collection in capillary tubes was the most appropriate procedure for field work and did not compromise the test accuracy [10,11]. However, studies directed toward the diagnosis of Chagas disease using this resource were not available.

The finding of this study that there is high accuracy and an excellent correlation between the PaGIA-Chagas results obtained with serum and those obtained with capillary blood-derived plasma may facilitate field work in epidemiological studies of chronic Chagas disease. Although PaGIA may not be practicable under field conditions, since special equipment is required to perform the assay, the potential to acquire a large number of fingerstick and capillary blood samples is likely to result in overall time savings and less complex field work.

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