

Diagnosis of *Trypanosoma cruzi* Infection Status using Saliva of Infected Subjects

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Abstract. Chagas disease has the highest prevalence of any parasitic disease in the Americas, affecting 6–7 million people. Conventional diagnosis requires a well-equipped laboratory with experienced personnel. The development of new diagnostic tools that are easy to use and adapted to the reality of affected populations and health systems is still a significant challenge. The main objective of this study was to measure *Trypanosoma cruzi* infection status using saliva samples of infected subjects. Blood and saliva samples from 20 *T. cruzi*-seropositive individuals and 10 controls were tested for *T. cruzi* infection using two different commercial serological tests. We have shown that detection of *T. cruzi* infection is possible using saliva samples, supporting the potential use of saliva to diagnose Chagas disease in humans. This method could provide a simple, low-cost but effective tool for the diagnosis of *T. cruzi* infection. Its noninvasive nature makes it particularly well suited for endemic areas.

INTRODUCTION

Chagas disease (CD) is a major public health problem in Latin America, affecting approximately 6–7 million people. In addition, travel and immigration patterns have increased the relevance of *Trypanosoma cruzi* infection outside of endemic areas.^{1–3} *Trypanosoma cruzi* can be transmitted to humans by reduviid insects that inhabit housing made of mud, thatch, and other natural materials in endemic areas⁴; by blood transfusion, organ transplant from infected donors,⁵ congenital route from mother to infant,⁶ and by ingestion of food or drink contaminated with infected triatomines.⁷

The diagnosis and management of CD still face multiple challenges. There are no preventive vaccines for human or veterinary use, and the two available treatments are not optimal as they are plagued by side effects and inconsistent efficiency.^{8–11} In addition, the diagnosis of this infection confronts several limitations. In the acute phase of the infection, diagnosis is based on the microscopic detection of trypomastigotes in blood. However, during the chronic phase of the infection, parasite persistence is low, and the detection of *T. cruzi* using this method is difficult and unreliable. Serology is the gold standard for *T. cruzi* infection diagnosis during the chronic phase of the disease. At least two different serological methods (preferably based on different antigens) are used to detect the presence of immunoglobulin G (IgG) antibodies against *T. cruzi* antigens. Currently, no single assay for the detection of chronic *T. cruzi* infection has shown enough sensitivity and specificity to be used alone for diagnosis.¹²

Chagas disease particularly affects poor rural and peri-urban areas of Latin America, where health-care access is limited. Developing new diagnostic tools which are easy to use and adapted to the reality of affected populations and health systems is still a substantial need.¹³ The purpose of this study

is to address this challenge by exploring the detection of *T. cruzi* infection status using saliva samples.

MATERIALS AND METHODS

Ethics statement. The study protocol was approved by the Ethics Committee of the Hospital Clinic of Barcelona and the Scientific Committee of the Barcelona Institute for Global Health (ISGlobal). Individual written informed consent was obtained from all study participants before the collection of samples.

Design and setting. The study was designed as a pilot project to detect *T. cruzi* infection status using the saliva of infected individuals. A total of 20 *T. cruzi*-seropositive individuals were enrolled in one group (GA) and 10 *T. cruzi*-seronegative individuals in another group (GB). GB was included as a negative control group. GA patients were originally from Latin American countries where CD is endemic. Among them, 10 received benznidazole treatment between 2005 and 2015 (all of them completed the treatment regimen) and 10 of them did not receive treatment during the length of the study. GB subjects came from different areas of the world.

Selection of subjects. Inclusion criteria: adult subjects (older than 18 and less than 50 years old); weight more than 40 kg, with serologic tests confirming or excluding *T. cruzi* infection. All the participants were recruited at the Center for International Health at the Hospital Clinic of Barcelona, Spain.

Participants. Subjects of both genders aged between 18 and 50 years were included in the study (Table 1).

Sample collection and procedures. *Blood samples.* Per individual 10 mL blood sample was collected for diagnosis of *T. cruzi* infection in serum. The samples were centrifuged at 1,600 × g for 10 minutes at room temperature.

Saliva samples. Subjects also provided 10 mL of unstimulated saliva samples. The saliva samples were centrifuged using ultrafiltration membranes (Amicon Ultra-15 devices) at 4,000 × g for 15 minutes at room temperature.

ELISA tests. To perform the serological test, 300 µL of concentrated saliva samples and 10 µL of blood serum were used. Two different commercial serological tests were used: BioELISA CHAGAS (Biokit S.A., Lliçà d'Amunt, Barcelona,

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TABLE 1
Age and gender of study participants

	Age	Gender	
GB	European controls	39	Female
		32	Male
		31	Male
		35	Female
		26	Female
	Endemic country controls	28	Female
		43	Male
		30	Female
		39	Male
		28	Female
GA	Untreated patients	38	Female
		31	Female
		44	Male
		44	Female
		42	Female
		39	Female
		44	Male
	Treated patients	42	Female
		34	Male
		33	Female
		54	Female
		46	Female
		43	Female
		50	Female
33	Female		
49	Female		
40	Female		
48	Female		
50	Female		
41	Female		

Spain) and DRG *Trypanosoma cruzi* IgG (DRG International Inc., Springfield, NJ).

Data analysis. Statistical analysis was performed by analysis of variance and unpaired *t* test using the GraphPad PRISM 5.0 software.

RESULTS

We first detected *T. cruzi* infection status with the current serological tests using enzyme-linked immunosorbent assay (ELISA) from Biokit. As expected, seropositive patients showed higher levels of IgG anti-*T. cruzi* antibodies compared with *T. cruzi*-seronegative subjects (Figure 1).

Before running ELISA on the saliva samples of *T. cruzi*-infected individuals, we tested whether a filtered and, therefore, a more concentrated saliva sample would exhibit higher sensitivity than an unfiltered sample. Filtered saliva samples showed higher levels of response to *T. cruzi* antigen than their unfiltered counterparts, although these differences were not statistically significant (Figure 2A). In addition, different volumes of filtered saliva samples were tested and the results showed higher sensitivity when using 300 µL compared with other volumes (Figure 2B).

Then, we tested 20 *T. cruzi*-seropositive and 10 *T. cruzi*-seronegative serum and saliva samples using the same test. The results are summarized in Figure 3. *Trypanosoma cruzi*-seropositive subjects presented higher levels of IgG anti *T. cruzi* antibodies compared with *T. cruzi*-seronegative subjects, using serum or saliva samples (Figure 3A). The ELISA results demonstrated consistency between serum and saliva

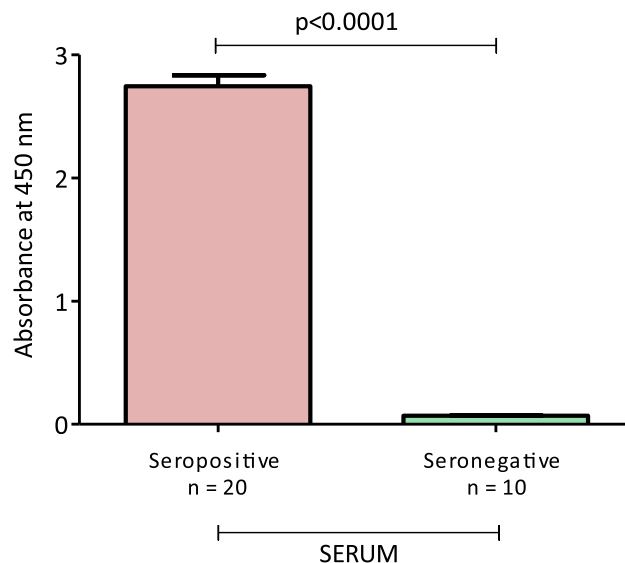


FIGURE 1. Serum immunoglobulin G response to *Trypanosoma cruzi* antigen in *T. cruzi*-seropositive and *T. cruzi*-seronegative subjects. This figure appears in color at www.ajtmh.org.

samples. The saliva test presented a specificity of 100%: all *T. cruzi*-seronegative individuals were negative using saliva as a sample. The sensitivity of the test was 70%: six of the 20 *T. cruzi*-seropositive individuals were negative using saliva as a source. Four of these subjects were previously treated with benznidazole and two of them were not previously treated. The ELISA's cutoff value was computed by using the usual cutoff formula of the form "mean +3 standard deviation of negative controls."

We also compared levels of *T. cruzi* antibodies for both saliva and serum of previously treated and untreated seropositive subjects. Differences in levels of serum IgG anti-*T. cruzi* antibodies were not found between treated and untreated individuals. Interestingly, we found differences in levels of *T. cruzi* antibodies between treated and untreated individuals using saliva (Figure 3B). However, these results were not statistically significant.

DISCUSSION

The most common method of diagnosing CD is detecting serum antibodies against parasite antigens mainly using ELISA tests. However, there are some limitations of using serum as a diagnostic sample. Blood collection is invasive, and it demands specially trained personnel.

Saliva has the potential to become a first-line diagnostic sample of choice. The use of this fluid for diagnostic purposes is increasing in popularity: it is easy to collect, store, and transport; it does not require highly trained personnel; and it is safer for medical staff to handle compared with other body fluids. In addition, it is noninvasive and the donation process is relatively stress free, minimizing donor discomfort. This can help improve access to diagnosis and treatment of people living in rural areas far from health-care centers. These characteristics make salivary diagnosis especially valuable for vulnerable populations. In addition, analysis of saliva may provide a cost-effective approach for the screening of large populations.^{14,15} Because of its many potential advantages,

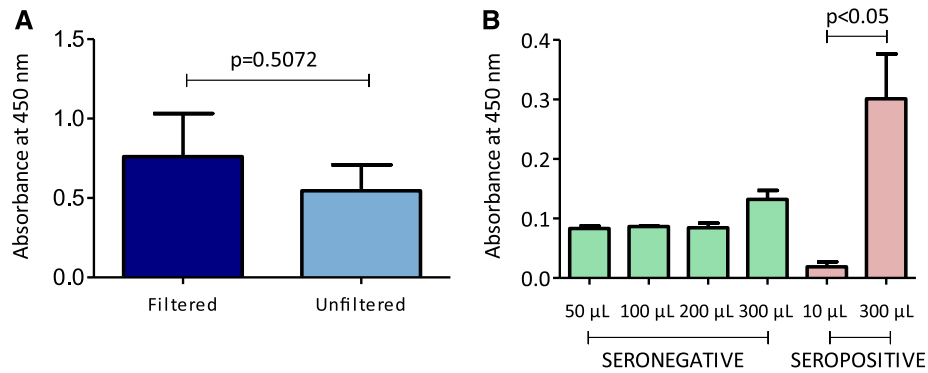


FIGURE 2. (A) Saliva immunoglobulin G (IgG) response to *Trypanosoma cruzi* antigen using ultrafiltration membranes. (B) Saliva IgG response to *T. cruzi* antigen in *T. cruzi*-seropositive and *T. cruzi*-seronegative subjects using different sample volumes. This figure appears in color at www.ajtmh.org.

salivary diagnosis provides an attractive alternative to more invasive, time-consuming, complicated, and expensive diagnostic approaches.

Pinho et al.¹⁶ explored this area of research in 1999 for *T. cruzi* infection, showing that detection of *T. cruzi* infection was possible using saliva samples. Our data confirm these findings, and thus support saliva as a possible source for the detection of CD.

Although our results have shown consistency between serum and saliva samples of *T. cruzi*-seropositive and *T. cruzi*-seronegative subjects, the low sensitivity of the ELISA test using saliva as a source of diagnosis could present a challenge. It is common to find low concentrations of molecules in saliva compared with blood.¹⁵ This weakness could be partially overcome using ultrafiltration membranes to concentrate saliva samples.

Our results have shown differences in the levels of IgG *T. cruzi* antibodies between previously treated subjects and untreated patients. Presently, the gold standard for evaluating treatment efficacy is the seroconversion of conventional serological tests, which may take years to decades to assess.¹⁷

Although this is a preliminary finding, saliva could play an important role in evaluating treatment efficacy.

CONCLUSIONS

Trypanosoma cruzi-specific salivary IgG detection provides a simple, low-cost but effective tool in the diagnosis of Chagas disease. The noninvasive nature of this method makes it particularly well suited to endemic areas. The saliva test presented a specificity of 100%. Unfortunately, the sensitivity of the test was lower: 30% of *T. cruzi*-seropositive individuals were negative using saliva as a diagnostic sample.

In the future, saliva could play a key role in the diagnosis of *T. cruzi* infection and evaluation of treatment efficacy. Additional studies exploring other salivary molecules such as IgA anti-*T. cruzi* levels could be helpful because of the high concentration of this immunoglobulin in saliva. More research is needed with larger sample sizes to further investigate this method, which has the potential to revolutionize the diagnosis and treatment of Chagas disease.

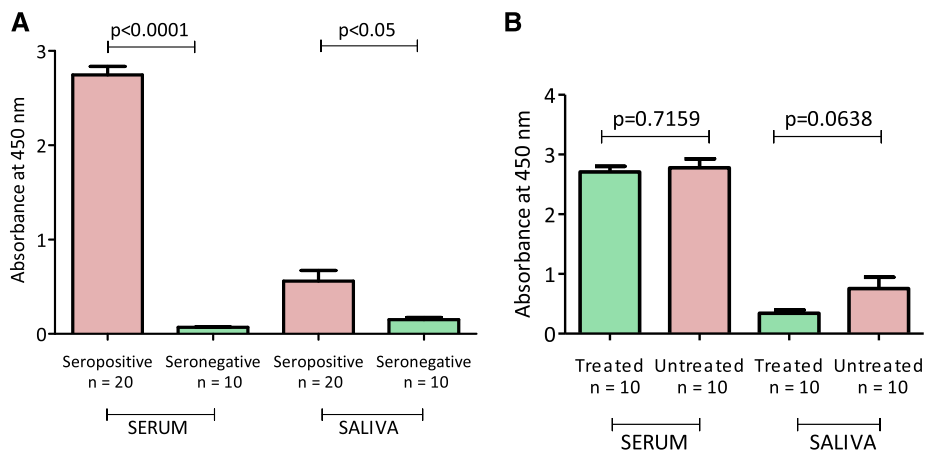


FIGURE 3. (A) Serum and saliva immunoglobulin G (IgG) response to *Trypanosoma cruzi* antigen in *T. cruzi*-seropositive and *T. cruzi*-seronegative subjects. (B) Serum and saliva immunoglobulin G response to *T. cruzi* antigen in *T. cruzi*-seropositive subjects previously treated with benznidazole and *T. cruzi*-seropositive untreated subjects. This figure appears in color at www.ajtmh.org.

Received February 22, 2017. Accepted for publication October 22, 2017.

Published online January 8, 2018.

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