Sensitivity and Specificity of an Operon Immunochromatographic Test in Serum and Whole-Blood Samples for the Diagnosis of Trypanosoma cruzi Infection in Spain, an Area of Nonendemicity

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Trypanosoma cruzi infection is an imported parasitic disease in Spain, and the majority of infected individuals are in the chronic phase of the disease. This study evaluated the sensitivity and specificity of the Operon immunochromatographic test (ICT-Operon; Simple Stick Chagas and Simple Chagas WB [whole blood]; Operon S.A., Spain) for different biological samples. Well-characterized serum samples were obtained from chagasic patients (n = 63), nonchagasic individuals (n = 95), visceral leishmaniasis patients (n = 38), and malaria patients (n = 55). Noncharacterized specimens were obtained from Latin American immigrants and individuals at risk with a clinical and/or epidemiological background: these specimens were recovered serum or plasma samples (n = 450), whole peripheral blood (n = 94), and capillary blood (n = 282). The concordance of the results by enzyme-linked immunosorbent assay and indirect immunofluorescence test was considered to be the “gold standard” for diagnosis. Serum and plasma samples were analyzed by Stick Chagas, and whole blood was analyzed by Simple Chagas WB. The sensitivity and specificity of the ICT–Operon in well-characterized samples were 100% and 97.9%, respectively. No cross-reactivity was found with samples obtained from visceral leishmaniasis patients. In contrast, a false-positive result was obtained in 27.3% of samples from malaria patients. The sensitivities of the rapid test in noncharacterized serum or plasma, peripheral blood, and capillary blood samples were 100%, 92.1%, and 86.4%, respectively, while the specificities were 91.6%, 93.6%, and 95% in each case. ICT–Operon showed variable sensitivity, depending on the kind of sample, performing better when serum or plasma samples were used. It could therefore be used for serological screening combined with any other conventional test.

Trypanosoma cruzi infection, or Chagas’ disease, is one of the major public health problems in Latin America and even in countries where the disease is not endemic (33). According to recent estimates, the disease affects about 10 million individuals living in areas of endemicity; however, its true scale in areas free of vector transmission is unknown (42).

In Spain, in recent years, an increase in the number of imported cases of T. cruzi infection has been reported as a result of increased migration from areas where the disease is traditionally endemic. Also, as a result of transmission that has occurred (i) through blood transfusion, (ii) through organ transplantation from infected donors, and (iii) from infected mothers to children during pregnancy or childbirth, T. cruzi infection has joined the list of autochthonous parasitic infections in this country. It is estimated that between 40,000 and 80,000 infected individuals may be residing in Spain; however, only 3,300 individuals have been diagnosed, along with more than 20 congenital cases and 6 cases of transfusional Chagas’ disease (1, 11–13, 23–25, 27–29, 34, 35, 39, 41).

Most of those affected are generally found to be in the chronic phase of the disease (25, 29). At this stage of the infection, anti-T. cruzi antibody detection is still the tool of choice for confirming suspected infection. Despite technological advances, there is still no “gold standard” test, so laboratory diagnosis is still based on the agreement between at least two different serological tests with different principles and antigens.

In Latin America, one of the best combinations recommended for diagnosing the infection is the enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence test (IFAT) binomial (30). A WHO technical report recommends a single ELISA, assumed to have approximately 99% sensitivity, for blood bank sample screening (40). Both ELISA and IFAT require specialized infrastructure and personnel, so their use in field studies is restricted. Immunochromatographic test (ICT) development has in part solved this problem, as ICTs are simple and can be run in less than 20 min.

In Spain, there are currently numerous tests in use for the detection of anti-T. cruzi antibodies (10). According to several studies conducted in both Latin America and Spain, tests based on ELISA are most appropriate for screening (40). However, the requirements of these tests do not allow their use in situ, even in well-developed areas. Furthermore, the resident infected population in Spain is an immigrant population experiencing employment difficulties that can cause frequent changes in residency. Under these circumstances, the possibility of undertaking rapid detection of anti-T. cruzi antibodies would therefore greatly facilitate the recruitment and monitoring of infected individuals who are unaware of their condition.

Given this context, our objective was to evaluate the sensitivity and specificity of the Operon immunochromatographic test (Sim-
pl/Stay Chagas and Simple Chagas WB [whole blood] using samples of serum, plasma, peripheral blood, or capillary blood.

**MATERIALS AND METHODS**

**Well-characterized samples.** Serum samples obtained from the bank of the Parasitology Department, Centro Nacional de Microbiologia, Instituto de Salud Carlos III (CNM-ISCIII), were used. These samples were characterized by in-house ELISA (Tc-ELISA) and in-house IFAT (Tc-IFAT): (i) 63 serum samples from individuals with Chagas’ disease, 19 of them with parasitemia nondetectable by PCR (ChPCR) and 44 with parasitemia detectable by PCR (ChPCR⁺), and (ii) 95 serum samples from seronegative individuals (nonchagasic samples). These samples were selected by taking into account the individuals’ clinical and epidemiological backgrounds. We did not include samples with indeterminate or discrepant serology. To assess cross-reactions, the following were included: (iii) 38 serum samples from individuals with visceral leishmaniasis (VL) and (iv) 55 serum samples from individuals with malaria. Serological reactivity was verified by in-house Leishmania IFAT and Plasmodium IFAT (Falciparum-Spot IF; bioMérieux, France), respectively. All these samples were evaluated during the same working week using the three tests for detecting anti-T. cruzi antibodies (the ICT from Operon [ICT-Operon], Tc-ELISA, and Tc-IFAT). Prior to selection, all samples were kept at −20°C. During testing, all samples were maintained at 4°C.

**Noncharacterized samples. (i) Population 1.** From June 2006 to June 2007, we analyzed 450 serum samples from individuals with an epidemiological background of suspected infection with T. cruzi. Samples were collected for detection of anti-T. cruzi antibodies by Tc-ELISA and Tc-IFAT at the Parasitology Department and included samples from (i) 300 blood donors, (ii) 25 pregnant Latin American women, and (iii) 125 Latin American immigrants, travelers, or aid workers who had either spent time or lived in areas of endemcity or who were individuals with Latin American ancestry on the mother’s side. The above-described classification was made using data provided by the transfusion center or the hospital of origin. The demographic data of this population were not analyzed, as they were incomplete. The rapid test and Tc-ELISA were performed on the same day, and the Tc-IFAT test was conducted during the same working week. During this period of time, all samples were stored at 4°C prior to testing.

(ii) **Population 2.** In a 4-month period (June to September 2006), we analyzed peripheral whole-blood samples from 94 individuals with a history of risk of infection by T. cruzi. The samples belonged to (i) 2 donors, (ii) 4 pregnant women, (iii) 81 immigrants, travelers, aid workers, or others with an at-risk background, and (iv) 7 neonates (newborn or up to 1 month old). The associated demographic data were not analyzed, as they were incomplete. The rapid test results were compared with those obtained by the conventional tests, Tc-ELISA and Tc-IFAT, in serum or plasma. The rapid test was performed on the day that the sample arrived at the Parasitology Department; Tc-ELISA and Tc-IFAT were run on separate days during the same week. During this time, serum and plasma samples were kept at 4°C.

(iii) **Population 3.** Between May 2008 and December 2009, capillary blood samples were taken by finger prick for anti-T. cruzi antibody detection using the rapid test. At the same time, a few drops of blood were collected in parallel on Whatman no. 1 filter paper for anti-T. cruzi antibody screening by conventional tests. Filter paper samples were air-dried and kept in self-sealing plastic bags at 4°C until Tc-ELISA and Tc-IFAT were run at CNM-ISCIII. Samples from 282 individuals were analyzed: 211 individuals from Bolivia, 29 individuals from Ecuador, and 42 individuals from other Latin American countries or individuals born in Spain with epidemiological risk factors. Within this population, 95 (33.7%) were male aged between 1 and 68 years (mean, 35 ± 11 years) and 187 (66.3%) were female aged between 3 months and 60 years (mean, 32 ± 10 years). Individuals with positive or indeterminate results were recalled for collection of a further blood sample to detect anti-T. cruzi antibodies by conventional serum testing. This study was performed within a public health and surveillance program on Chagas’ disease conducted by the Tropical Medicine Unit at the Ramón y Cajal Teaching Hospital (TMU-RCH) (26). To define the state of compromise and decide on further treatment, TMU-RCH conducted a thorough clinical assessment not described in this paper. This study was approved by the Ethics Committee of the Ramón y Cajal Hospital in Madrid, Spain.

**Conventional tests for detection of anti-T. cruzi antibodies. (i) Tc-ELISA.** Tc-IFAT was performed according to the protocol described by Camargo (3) with some modifications (10). Briefly, epimastigotes from stationary-phase cultures of three strains of T. cruzi (strains Mc, T, and DM28) were used. Ten microliters of a suspension containing 5 × 10⁶ parasites/ml of a proportional mixture of each strain was placed in well. Four 2-fold dilutions of the sample were prepared from the 1/20 dilution stock. As conjugate, human anti-IgG labeled with fluorescein (bioMérieux, France) was used. A sample was considered positive when it presented an antibody titer of ≥1/40.

(ii) **Tc-ELISA.** Tc-ELISA was performed following the protocol described by Flores-Chavez et al. (10). In summary, soluble antigen was prepared from cultures of the same T. cruzi strains used for Tc-IFAT. Nunc Maxisorp plates were sensitized with 1.5 µg/well of the proportional mix of each soluble antigen diluted in 0.05 M carbonate buffer (pH 9.6). After blocking with phosphate-buffered saline (PBS) supplemented with 3% bovine serum albumin (BSA) and 0.1% Tween 20, plates were washed 5 times with PBS plus 0.1% Tween 20 (PBS-T). Control and test sera were diluted 1/100 in PBS-T with 0.1% BSA (PBS-T-BSA). To reveal the antigen-antibody reaction, a biotin-streptavidin system (Southern Biotechnology Associates, Inc.) was used. ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); Sigma] diluted in 0.05 M phosphate-buffered citrate (pH 5.0) with 0.03% sodium perborate (Sigma) was used as the substrate. The reaction was stopped with 5% SDS solution. The absorbances were measured at a wavelength of 405 nm. The cutoff (CO) was defined as the mean optical density (OD) of 53 negative controls plus 4 times the standard deviation. The gray zone was defined as ±20% of the CO. To compare antibody levels, we calculated the reactivity index (sample OD/cutoff).

(iii) **ICT-Operon.** The commercial ICT-Operon (Operon SA, Spain) uses a recombinant multiepitope protein (Pep2-TcD-TcE-SAPA) as antigen and comes in two formats: (i) as a dipstick designed to detect anti-T. cruzi antibodies in serum or plasma (Simple Stick Chagas) and (ii) as a cassette designed for serum, plasma, or whole-blood samples (Simple Chagas WB) (Fig. 1). In this study, the stick format was used to detect anti-T. cruzi antibodies in serum and plasma samples and the cassette
TABLE 1 Reactivity of well-characterized serum samples by Tc-ELISA, Tc-IFAT, and ICT-Operon

| Clinical status       | Total | Tc-ELISA | Tc-IFAT | ICT-Operon |
|-----------------------|-------|----------|---------|------------|
| Chagasic patients     | 63    | 63       | 63      | 63         |
| Nonchagasic or healthy individuals | 95    | 0        | 0       | 2          |
| Leishmaniasis patients| 38    | 18       | 23      | 0          |
| Malaria patients      | 55    | 0        | 0       | 15         |

device was used for whole-blood samples. The immunochromatographic test was performed according to the manufacturer’s instructions. In short, each sample of serum or plasma was diluted 1/15 in dilution buffer in a 1.5-ml microtube. After homogenization, a dipstick was placed vertically in each tube and left for 10 min. When the sample was whole blood, a 25-μl aliquot was placed in the appropriate window of the cassette. After adding dilution buffer, the device was kept on a flat surface for 10 min. In both cases, the reading and interpretation of the results were done within 30 s of the time set by the manufacturer.

The test was valid when a blue line appeared in the area marked with the letter C (test control); the absence of a reaction in this area implied the need for immediate repetition of the test. The sample was considered reactive for *T. cruzi* when a red/violet line appeared on the site marked with the letter T (test result); its absence indicated that the sample was not reactive. Under laboratory conditions, the optical assessment was performed by two observers to obtain a consensus result.

Detection of antibodies in blood samples on filter paper. Detection of antibodies in blood samples on filter paper was performed as previously described by Machado-Coelho et al. (21), with some modifications. Briefly, for each individual/sample, 2 discs (5-mm diameter) of blood-impregnated filter paper were placed in 96-well plates and 140 μl of PBS-T-BSA was added (dilution factor, 1/20). After 1 h incubation at room temperature and continued shaking, 50 μl of the corresponding eluate was used to prepare four further 2-fold dilutions (1/80 to 1/640) in PBS-T-BSA. One hundred microliters of each dilution was used for the Tc-ELISA. Alternatively, 15 μl of each eluate was used to prepare 1/40 and 1/80 dilutions in PBS; 10 μl of each dilution was then used to perform the Tc-IFAT. As well as the controls used on the respective tests, positive and negative blood samples impregnated in filter paper were prepared experimentally and were included in each protocol to assess interassay variability. The OD readings of the 1/160 and 1/320 dilutions were used to define reactivity (positive or negative). The choice of one or another dilution was based on the results obtained in the same dilutions of blood-impregnated filter paper of the positive and negative controls. Tc-IFAT results were interpreted as described above (cutoff titer, 1/40).

Data analysis. Data were entered into an Excel (Microsoft) spreadsheet, and statistical analysis was performed by using SPSS Statistics (version 17.0) software. The sensitivities and specificities of the serological tests were calculated using Epidat (version 3.1) software (www.sergas.es) and Bayes Latent Class Models (BLCM) software (version 1.4) (8).

An individual was considered seropositive for infection with *T. cruzi* when both conventional tests, Tc-ELISA and Tc-IFAT, were positive. Individuals who presented negative results in conventional tests were considered seronegative. Any individual showing disagreement between the two tests was considered serodiscrrent, and these individuals were included in the population of seronegative subjects in order to calculate the sensitivities and specificities of the serological tests using the Epidat program.

Currently, none of the available tests are considered to be a true gold standard. Therefore, to estimate the performance of tests in the absence of a gold standard, a latent class model was used. This model considered the true disease status as a latent (unobserved) variable to determine the observed test results and provided estimates of sensitivity and specificity for each test. For this analysis, we assumed the independence of the three tests and used truncated distributions for sensitivity and specificity, taking into account the results reported above (10).

RESULTS

Reactivity and performance of Operon immunochromatographic test using well-characterized samples. All serum samples (100%, n = 63) from chagasic patients and 2 (2.1%, n = 95) from nonchagasic individuals were reactive by Simple Stick Chagas. None (0%, n = 38) of the serum samples from individuals with VL were reactive; in contrast, 15 (27.3%, n = 55) serum samples from malaria patients were positive (Table 1; Fig. 2).

Simple Stick Chagas sensitivity in previously characterized samples was 100% (95% confidence interval [CI], 99.2% to 100%), and the specificity was 97.9% (95% CI, 94.5% to 100%). When the results obtained for sera from individuals with visceral

FIG 2 Levels of anti-*T. cruzi* antibodies using Tc-ELISA in characterized samples versus Tc-IFAT (A) and versus Simple Stick Chagas ICT-Operon (B). The dashed line represents the threshold of positivity for Tc-ELISA. Samples from the following patients were tested: ChPCR−, Chagasic patients with parasitemia nondetectable by PCR (n = 19); ChPCR+, Chagasic patients with parasitemia detectable by PCR (n = 44); nonchagasic patients (n = 95); MAL, malaria patients (n = 55); VL, visceral leishmaniasis patients (n = 38). White circles, negative Tc-IFAT or ICT result; gray circles, reactive Tc-IFAT titer of 1/20; black circles, positive Tc-IFAT or ICT result.
leishmaniasis and malaria were included in the analysis, specificity decreased to 91% (95% CI, 86.6% to 95.3%).

**Reactivity of Operon immunochromatographic test in non-characterized samples.** In population 1, 10.4% (47/450) of samples were reactive by both Tc-ELISA and Tc-IFAT (positive samples). Only 1.6% (7/450) of cases showed discordant results in conventional tests (indeterminate serology). The remaining samples, 88% (396/450), showed no reactivity to total antigens of *T. cruzi* (negative samples). One hundred percent (47/47) of positive samples and 7.6% (30/396) of negative samples were reactive by the rapid test (Table 2 and Fig. 3A).

In population 2, 43 (93.5%) out of 46 blood samples reactive by the rapid test were confirmed by Tc-ELISA and Tc-IFAT in serum or plasma. Four (8.5%) out of 47 samples which were positive by conventional serology were negative by Simple Chagas WB (Table 2; Fig. 3A).

In population 3, of the 282 tests performed, the Simple Chagas WB test was reactive in 51 cases. One of the reactive samples was from a 3-month-old girl who presented discrepant conventional serology, a negative Tc-ELISA result, and a Tc-IFAT titer of 1/40. Of the other reactive cases, 76% (38/50) showed consistent positive reactivity by conventional tests. Of the 231 people with a negative ICT result, 6 showed consistent positive reactivity with Tc-ELISA and Tc-IFAT and 1 presented discrepant results. Taking concordance between Tc-ELISA and Tc-IFAT as the gold standard, 86.3% (38/44) of these seropositive individuals were reactive with Simple Chagas WB (Table 2; Fig. 3C).

**Confirmation of results obtained on filter paper, population 3.** Of the individuals called in order to confirm the results obtained by the rapid test in situ and the conventional tests on filter paper-embedded blood, 34 individuals returned. Confirmation was done by conventional tests on serum samples. Thus, of the 38 cases that showed ICT-positive (ICT+), Tc-ELISA-positive (Tc-ELISA+), and Tc-IFAT-positive (Tc-IFAT+) results, 25 attended the second appointment. Of these, 24 presented positive concordant serology, while the remaining person showed no reactivity (result obtained 2 years later). Only 3 of the 12 individuals who had ICT+, Tc-ELISA-negative (Tc-

**TABLE 2 Reactivity of noncharacterized samples by Tc-ELISA, Tc-IFAT, and ICT-Operon**

| Result                  | No. of samples |
|-------------------------|----------------|
| Tc-ELISA                | Population 1 (serum or plasma) | Population 2 (peripheral blood) | Population 3* (capillary blood) |
| + + +                   | 47             | 43             | 38             |
| + + –                   | 0              | 4              | 6              |
| – + +                   | 0              | 0              | 1              |
| – – +                   | 30             | 3              | 12             |
| + – –                   | 2              | 0              | 1              |
| – – –                   | 5              | 0              | 0              |
| – – –                   | 366            | 44             | 224            |
| Total                   | 450            | 94             | 282            |

* The results for Tc-ELISA and Tc-IFAT are those obtained on blood-impregnated filter paper.

![Figure 3](https://example.com/fig3.png)

**FIG 3** Comparison of levels of anti-*T. cruzi* antibodies by Tc-ELISA versus Tc-IFAT and ICT-Operon. (A) Population 1, serum or plasma samples from immigrants from areas of endemicity and travelers/aid workers staying in areas of endemicity (n = 125), pregnant women (n = 25), and at-risk donors (n = 300); (B) population 2, peripheral blood samples from individuals with epidemiological background (n = 94); (C) population 3, capillary blood samples from Latin American immigrants and those born in Spain from Latin American parents (n = 282). White circles, negative ICT result; black circles, positive ICT result.
TABLE 3 Sensitivity and specificity of Tc-ELISA, Tc-IFAT, and ICT-Operon in different populations and sample types based on Bayesian latent class analysis

| Population (sample) | Test                  | Sensitivity Estimate (%) (95% CI) | Specificity Estimate (%) (95% CI) |
|---------------------|-----------------------|----------------------------------|----------------------------------|
| 1 (serum and plasma)| Tc-ELISA              | 100                              | 98.8                             |
|                     | Tc-IFAT               | 100                              | 96.6                             |
|                     | ICT-Operon            | 100                              | 92.6                             |
| 2 (peripheral blood)| Tc-ELISA              | 98.2                             | 95.6                             |
|                     | Tc-IFAT               | 98.1                             | 99.2                             |
|                     | ICT-Operon            | 91.8                             | 93.7                             |
| 3 (capillary blood) | Tc-ELISA              | 85.5                             | 99.8                             |
|                     | Tc-IFAT               | 98.5                             | 98.4                             |
|                     | ICT-Operon            | 86.1                             | 94.8                             |

ELISA−), and Tc-IFAT-negative (Tc-IFAT−) results attended the second appointment. Of these, 2 remained Tc-ELISA− and Tc-IFAT−, while the third presented discrepant results which were maintained during the first year of follow-up. In a checkup performed 23 months later, the patient began to show concordant positive results and continued to do so in subsequent tests. Four of the six cases who were ICT negative (ICT−), Tc-ELISA−, and Tc-IFAT− returned for confirmation of the results on serum. All of them remained Tc-ELISA− and Tc-IFAT−. The one case with discrepant conventional serology (ICT−, Tc-ELISA−, Tc-IFAT−) did not attend the second appointment. The 3-month-old girl who presented ICT+, Tc-ELISA−, and Tc-IFAT− recorded a concordant negative serology 5 months later (at 8 months old). Five people who showed optical density readings near the cutoff were also called in for collection of a second blood sample, but only one attended the appointment and presented Tc-ELISA+ and Tc-IFAT+ (results obtained 2 months later).

DISCUSSION

Chagas’ disease has been classified as a disease of the poor, since its presence is usually associated with rural areas lacking basic services, education, and health care. Asymptomatic infected immigrants in search of better job opportunities can unintentionally carry *T. cruzi* from remote areas of endemicity. This has significantly increased the number of infected individuals in Spain. Since a significant proportion of individuals in the affected population are unaware of their infection, Chagas’ blood screening programs are essential, even in regions of nonendemicity or in areas free of vector transmission. In Spain, Royal Decree 1088/2005 (22a) requires all blood transfusion centers to run a serological test to rule out at-risk donations. Thus, the detection of anti-*T. cruzi* antibodies to rule out at-risk donations has contributed to reductions in the risk of transmission by this route, since no new cases have been reported.

Another important route of transmission is transplacental, which cannot be interrupted. One way to identify children with congenital infection is to carry out serological screening of pregnant women from areas of endemicity or deemed to be at risk during either pregnancy or childbirth and to perform follow-up tests of their children. Currently, control of vertical transmission is legislated only in the autonomous communities of Valencia and Catalonia (7, 9). In the rest of Spain’s self-governing regions (autonomous communities), detection of vertical transmission depends on the initiative of the different health professionals involved (14).

As mentioned above, the affected population often has job-related circumstances that do not facilitate their follow-up. Therefore, the rapid detection of anti-*T. cruzi* antibodies on first contact with the at-risk population is essential for planning a monitoring schedule that is dependent on patients’ availability. Only immunochromatographic tests provide speed and simplicity under some limited circumstances.

Currently, there are several rapid tests for the detection of anti-*T. cruzi* antibodies (10, 15, 17, 19). Among them, the Chagas Stat-Pak has been widely used and validated for serological screening programs both in the field and in blood banks for serum, peripheral blood, and umbilical cord blood samples (4–6, 19, 31, 37, 38). According to these studies, the sensitivity and specificity of Chagas Stat-Pak are fairly adequate in Bolivia.

Although the population most affected in Spain and other European countries is predominantly Bolivian (16, 23, 25, 29), the largest migrant Latin American populations come from Ecuador, Colombia, and Peru. In addition to the variability that this entails, an increase in tourist travel and/or associated family visits is exposing these individuals to many different environments where they present a risk.

In this context, the present study has assessed the performance of the Operon immunochromatographic test for detection of anti-*T. cruzi* antibodies in characterized and noncharacterized clinical samples under laboratory conditions and in the field, using serum or plasma, peripheral blood, and capillary blood.

The Operon immunochromatographic test performance with serum or plasma samples analyzed under laboratory conditions was extremely good (100% sensitivity) in both well-characterized and noncharacterized samples (population 1). Also, if we compare the reactivity patterns of Tc-ELISA and Tc-IFAT on the one hand and Tc-ELISA and ICT-Operon on the other, it can be seen that the pattern of reactivity in both cases overlaps (Fig. 2). Therefore, whenever a serum or plasma sample is used under laboratory conditions, the combination of a *T. cruzi* ELISA with the Operon immunochromatographic test (Simple Stick Chagas) could be useful for diagnosis of infection by *T. cruzi* in the absence of a *T. cruzi* IFAT.

On the other hand, ICT-Operon performance on peripheral blood (population 2) was poorer (92.1%). Three out of the four peripheral blood samples not recognized by the rapid test were from newborns that later proved not to be infected. The infants’ results were not excluded from the analysis of sensitivity and specificity, as at that age they usually have IgG antibody levels similar to those detected in their mothers. In fact, all the newborns showed positive concordant results by the two conventional tests, as was expected. Despite this, although antibody detection in the peripheral blood of newborns indirectly reflects the level of maternal antibodies, the antibodies may show a different pattern of recognition of certain epitopes which is not apparent when using total antigens (22).

After analyzing the results of the rapid test in capillary blood (population 3) and under field conditions, the sensitivity was found to be even lower (86.4%), although it was within the estimated confidence interval of our previous study, using serum or plasma (10). In a similar study using Simple Chagas WB in capillary blood samples, no false-negative results were obtained, al-
though it should be mentioned that the number of individuals with a confirmed diagnosis was smaller (n = 6) (18). In our experience, under laboratory conditions, using the Operon immunochromatographic test with serum or plasma samples has enabled the identification of infected individuals within a high-risk population, i.e., the true positives. Under field conditions, even with trained staff, technical problems that affect the reading and interpretation of results can occur. These drawbacks cannot always be controlled and can lead to poor concordance with the results of conventional tests.

The variability in the sensitivity of the Operon immunochromatographic test observed is a disadvantage not solely limited to this rapid test. Other authors have previously reported similar results with Chagas Stat-Pak (6, 37, 38), which were associated with the genetic variability of T. cruzi isolates in different geographical areas. Just as the performance of conventional tests would be conditioned by the preparation of the antigen from distinct strains circulating in different regions of endemicity, it is postulated that tests based on recombinant antigens would also have variable sensitivity depending on the geographical area of study (32, 38). Most studies agree that Chagas Stat-Pak has very good sensitivity when used for serological screening of the Bolivian population (6, 37, 38). In our population, 73% of the subjects were from Bolivia. In fact, 100% of the individuals with a confirmed diagnosis were from Bolivia, so the low sensitivity observed in blood samples cannot be attributed to geographical variation.

It should not be forgotten that the course of infection by T. cruzi is complex, and we cannot accurately determine at what stage of infection a patient presents. Without knowing the precise moment of contact with the parasite, the status of the parasitic inoculum, or the individual’s immune status, we cannot ascertain whether or not a person is in acute phase (window period) or if the person has a recent (less than 6 months) or chronic infection. Thus, the course of the acquired humoral response, depending on the Chagas' disease stage, will affect the efficiency of a specific serological tool. Therefore, when studying the performance of the test using well-characterized clinical samples, the categories infected and uninfected can be clearly differentiated, and it can even be established whether or not these individuals have circulating parasitemia (Fig. 2). However, in the daily routine, we always find at least four categories: seropositive results, seronegative results, serodiscordant results, and results close to the cutoff. In our experience, in the two last categories, the definition of the condition of an individual is established after a 1-year follow-up: after 6 months, discrepancies and indeterminate results usually disappear; only a small number of individuals show positive seroconversion (12). In this regard, it should be noted that in population 3, negative serocconversion was observed in a person from Mexico and positive serocconversion was observed in a person from Bolivia. Initially, both cases exhibited antibody levels close to the cutoff: just above in the first case and just below in the second.

Moreover, although negative serocconversion after treatment may occur in the long term, it has been reported that recombinant antigens are more efficient in showing the drop in specific anti-T. cruzi antibodies after treatment (36). Another major feature observed elsewhere after years of posttreatment follow-up was various antibody levels in conventional tests (20). These authors report the case of a patient who, before treatment, had a positive ELISA value 2.5 times higher than the cutoff value and IFAT titers of 1/1,280; in a control carried out 19 years after treatment, the patient showed ELISA results between 1 and 1.2 times the cutoff and IFAT titers between 1/20 and 1/40. In our study population 3, of the six false-negative results by the rapid test, there was evidence of prior treatment in only one case. However, we cannot rule out the possibility that the rest had been treated in their country of origin, since administration of the treatment is not currently certified.

Finally, in terms of specificity, it is important to note that the Operon test does not cross-react with sera from patients with visceral leishmaniasis. These individuals generally show a high degree of polyclonal activation that causes strong cross-reactivity in conventional tests for serological diagnosis of infection with T. cruzi (Fig. 2). Therefore, the antigen which is used to prepare the ICT may be useful in ruling out possible cross-reactivity with Leishmania infantum, the causative agent of visceral leishmaniasis in the Mediterranean Basin. In contrast, unlike other tests based on T. cruzi recombinant antigens, the number of false-positive results in samples from malaria patients was striking (10). These samples were selected from individuals who had acquired the Plasmodium infection in Africa and had no history of travel to Latin America, so their exposure to T. cruzi was ruled out. However, we should mention that exposure to other trypanosomatids was not ruled out. In this study, we have not fully considered the cross-reactivity with Plasmodium spp. Further studies are needed to assess the real impact of these false-positive results in areas of coendemicity.

In conclusion, the results suggest that the Operon immunochromatographic test is more useful in serum or plasma samples than in blood samples. This test could be useful as a first choice, preferably using serum or plasma samples. However, to achieve a good serological screening for T. cruzi infection, all samples analyzed by the rapid test should, preferably, also be analyzed with a conventional test to complement its principle. Nevertheless, further studies on a higher number of T. cruzi-infected individuals should be performed in order to confirm these results.

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