Dried Blood as an Alternative to Plasma or Serum for Trypanosoma cruzi IgG Detection in Screening Programs

Africa Holguín, Francesco Norman, Letícia Martín, María Luisa Mateos, Jesús Chacón, Rogelio López-Vélez, José A. Pérez-Molina

HIV-1 Molecular Epidemiology Laboratory, Microbiology Department, IRYCIS-Hospital Ramón y Cajal and CIBER-ESP, Madrid, Spain; Tropical Medicine, Infectious Diseases Department, IRYCIS-Hospital Ramón y Cajal, Madrid, Spain; Microbiology Department, IRYCIS-Hospital Ramón y Cajal, Madrid, Spain

Trypanosoma cruzi serological screening is recommended for people potentially exposed to this parasite in countries where Trypanosoma cruzi is endemic and those where it is not endemic. Blood samples on filter paper may be a practical alternative to plasma/serum for antibody detection. Using the Architect Chagas assay, we detected the presence of IgG against T. cruzi in matched serum and dried blood spots (DBS) collected from 147 patients residing in Madrid, Spain, who had potential previous exposure to T. cruzi. The κ statistic for the DBS/serum proportion of agreement for the detection of antibodies against T. cruzi was 0.803, considering an S/CO (assay result unit; chemiluminescent signal from the sample [S] divided by the mean chemiluminescent signal for the three calibrators used in the test [CO]) cutoff value of ≥1.00. The relative sensitivity of the Architect test using DBS increased from 95.2% to 98.8% when the cutoff was lowered from ≥1.00 to ≥0.88, while the relative specificity decreased from 84.1% to 71.6%. Overall, the median S/CO values for DBS were significantly lower than those for serum (2.6 versus 6.5; P < 0.001). Discrepancies that occurred with the use of DBS included 10 false positives (with low S/CO values in 9 cases [median, 2.13]) and 4 false negatives, with mean S/CO values of 0.905 (gray zone). Using DBS plus a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) may be a simple and reliable method for detecting IgG against T. cruzi when blood sampling by venipuncture is not feasible. This method may also reduce the false-negative rates observed with some rapid diagnostic tests. The lower relative sensitivity compared to the reference method may be increased by lowering the optical density threshold.

The protozoan Trypanosoma cruzi, the etiological agent of Chagas disease, has been affecting humans in remote rural areas of countries of endemicity for at least 9,000 years (1). Only recently, due to mobile populations, Chagas disease has emerged as a public health problem, not only in areas of the American continent where the disease is traditionally endemic, but also in other American countries such as the United States and Canada, as well as Japan, Australia, and a number of European countries (2). In 2005, the estimated prevalence of T. cruzi infection in the American continent was 8 to 10 million (3), with an incidence of chronic infection of 8 per 100,000 population for vectorial cases (n = 41,200) and 130 per 100,000 births for congenital cases (n = 14,385). The risk of congenital transmission from an infected mother ranged from 1% to 10%, and the overall mortality rate was 0.0023% (12,500 deaths per year) (4). The majority of infected individuals in Europe are immigrants who acquired T. cruzi in their countries of origin; there are an estimated 68,000 to 123,000 cases of the infection, with an estimated annual incidence of congenital transmission between 0 and 3 cases per 1,000 pregnancies in women from countries of endemicity (5, 6). In Europe, only a small proportion of cases had actually been diagnosed by the year 2009 (around 4,300 cases diagnosed; the majority, 89%, detected in Spain) (6). Thus, the estimated index of underdiagnosis was between 94% and 96%.

If acute infection is not recognized and treated, patients enter the chronic phase and have a 30% to 40% risk of developing visceral involvement after approximately 10 to 30 years (1). Physicians in countries where Chagas disease is not endemic may now be faced with a type of cardiomyopathy and an esophageal/intestinal disease with which they may be unfamiliar. During the chronic phase of T. cruzi infection, parasitemia is scarce and diagnosis is based on serological tests, such as the indirect immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assays (ELISAs), or indirect hemagglutination (7). For a patient to be considered infected, two positive results must be obtained with two serological tests using different antigens, although for screening purposes an ELISA-based assay can be used as a single test for the detection of infected patients (8). In settings where blood sampling by venipuncture is not feasible (field studies or targeted community programs for migrants), rapid diagnostic test (RDTs) could be an option. However, these tests must have their sensitivity improved, given the significant proportion of false negatives reported (1 to 14%) (9–11).

Blood samples obtained by finger puncture and collected on filter paper (dried blood spots [DBS]) are an inexpensive and practical alternative to plasma obtained by venipuncture for serological diagnostic techniques. DBS are easy to transport, without the need for cold chains or complex equipment. The utility of DBS for the diagnosis of infectious diseases and for genetic and serological testing has been known for years (12). Our objective was to test the proportion of agreement between the results obtained in serum and DBS samples using the Architect assay (Abbott) for screening for T. cruzi infections. This assay has shown high levels of specificity (99.99%) and sensitivity (99.85%) that are superior

Received 12 April 2013 Returned for modification 6 May 2013 Accepted 31 May 2013
Published ahead of print 5 June 2013
Address correspondence to José A. Pérez-Molina, jose.perezmolina@gmail.com.
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/CVI.00221-13
to those of other commercial ELISAs, along with excellent precision (13).

**MATERIALS AND METHODS**

**Study population and specimens.** This was a prospective observational study performed at the Ramón y Cajal University Hospital, from May 2011 to March 2012. All adult immigrants from Central/South America who attended our unit (with or without a previous diagnosis of *T. cruzi* infection), as well as those Spaniards who had been potentially exposed to *T. cruzi* infection due to travel or residence in Latin American countries where *T. cruzi* infection is endemic, were asked to participate. Two parallel samples were obtained by venipuncture (serum sample) and finger puncture (DBS) for all enrolled patients. The local ethics committee approved the study protocol (protocol CHAGAS-DBS-01, Ver 1.0; April 15, 2011).

DBS were obtained following finger puncture by adding 4 drops of blood to fill two 1.1-cm-diameter circles on 903 filter paper cards (Schleicher & Schuell BioScience GmbH, Barcelona, Spain). Cards were dried overnight at room temperature and stored at 80°C until processing (after a median of 1.5 months; range, 7 days to 4 months). Two 1.1-cm paper disks with dried blood were eluted with 300 μl of phosphate-buffered saline (PBS) buffer and incubated overnight with gentle rotation at room temperature. After the rest of the Whatman paper was removed by centrifugation, the supernatant was processed according to the Architect Chagas assay manufacturer’s instructions. Serum samples were also stored at −80°C immediately after collection and were analyzed within a maximum period of 3 weeks.

**Detection of Trypanosoma cruzi IgG antibodies.** The presence of IgG antibodies against *T. cruzi* was determined in both sera and eluted dried blood using the Architect Chagas assay (Abbott Laboratories, Germany), which uses four *T. cruzi* recombinant antigens. This assay has been licensed by the U.S. Food and Drug Administration (FDA) as a single test for screening blood donors for Chagas disease and has also been used in the clinical setting for routine screening. This fully automated instrument platform has previously demonstrated high specificity (99.99%) and sensitivity (99.85%) with excellent precision using serum or plasma (13). The result unit for the assay was S/CO (chemiluminescent signal from the sample [S] divided by the mean chemiluminescent signal for the three calibrators used in the test [CO]). Specimens with S/CO values of ≥1.00 (cutoff value for serum) were considered reactive for antibodies to *T. cruzi*, and specimens with S/CO values from ≥0.8 to 0.99 (in the gray zone) and those with S/CO values of <0.8 were considered nonreactive (13). An IFAT was used to confirm reactive specimens (in-house method performed at the National Microbiology Center Carlos III, Madrid, Spain) (14).

**Statistical analysis.** Sample size was estimated assuming a *T. cruzi* infection prevalence in our study population of around 31% (15) and an expected concordance index between serum and DBS of 90% (κ statistic, 0.90). For the lower limit of the 95% confidence interval (CI) of the κ index estimation to be ≥0.8, 151 patients had to be included.

Results for both serum and DBS were interpreted according to the Architect Chagas assay manufacturer’s cutoff value (S/CO value of ≥1.00). An appropriate cutoff value for the DBS technique was then determined by using a receiver operating characteristic (ROC) curve. Since our aim was to assess concordance of results using a validated serological assay when samples were obtained by two different methods (standard venipuncture versus DBS), we used the terms relative sensitivity and relative specificity, taking serum sample results as the reference. Relative sensitivity (true positives/total positives) versus one minus the relative specificity (false positives/total negatives) were plotted for all potential S/CO points for the DBS, taking serum sample results as the reference method.

Qualitative variables were expressed as absolute frequencies and percentages and quantitative variables as the median and interquartile range (IQR). Categorical variables were compared using the chi-square or Fisher exact test and continuous variables using the t test or Mann-Whitney U test. Differences were considered significant at a P value of <0.05. The SPSS (Chicago, IL) software package version 15.0 was used for statistical analysis.

**RESULTS**

Data on 147 of the 151 patients included in the study were analyzed. Data for three patients were excluded from analysis due to lack of matched samples and for one patient because of missing epidemiological information. The study population was mainly of Bolivian origin (77%) and female sex (66%), with a median age of 37 years and a median time from arrival to Spain of 81 months (Table 1).

Overall, for each matched samples, serum S/CO values were higher than those obtained with DBS (Fig. 1); the median value for DBS was 2.6 (25th to 75th percentile [P25–P75], 0.66 to 7.18), while the median value for serum was 6.5 (P25–P75, 0.04 to 10.50) (P < 0.001). The κ statistic for the proportion of agreement between DBS and serum samples for the detection of antibodies against *T. cruzi*, considering the cutoff value of ≥1, was 0.803 (95% CI, 0.705 to 0.901) (Table 2).

Most of the discrepancies in results were due to false positives with the DBS samples (n = 10) (Table 2). The S/CO values for 9 of these subjects were low (median, 2.13; range, 1.1 to 2.8), with four values near the gray zone (1.1, 1.18, 1.21, and 1.69). One subject showed a clear positive S/CO value of 5.75. In contrast, all 10 serum sample values for these patients were clearly negative (median, 0.035; range, 0.02 to 0.62), and the IFAT results for all were also negative. Four false-negative results were observed with DBS sampling, with S/CO values clearly below the figures obtained with serum samples (DBS median, 0.905 [range, 0.65 to 0.96] versus serum median, 4.85 [range, 3.5 to 6.37]). Of note, three of the values were in the gray zone (0.89, 0.92, and 0.96). The four

| Characteristic | Value for patients with: |
|---------------|--------------------------|
|               | *T. cruzi*-positive serum samples (n = 84) | *T. cruzi*-negative serum samples (n = 63) | P value |
| Sex (n [%])   | 56 (57.7) | 41 (42.3) | 0.84 |
| Female        | 28 (56.0) | 22 (44.0) | 0.001 |
| Male          | 37 (32–45) | 36 (31–42) | 0.27 |
| Median (IQR) age (yr) | 84 (56.4) | 33 (52.4) | <0.001 |
| Country of origin (n [%]) | 1, 0, 2, 0, 0, 0, 2, 0, 0, 0 | 81 (100) | 93 (90–103) | 0.40 |
| Bolivia       | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 81 (96.4) | 33 (52.4) | 0.40 |
| Brazil        | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 1 (1.2) | 10 (15.9) | 0.001 |
| Colombia      | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 (1.6) | 1 (1.6) | 0.001 |
| Ecuador       | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 1 (1.2) | 10 (15.9) | 0.001 |
| Guatemala     | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 (1.6) | 1 (1.6) | 0.001 |
| Honduras      | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 (1.6) | 1 (1.6) | 0.001 |
| Paraguay      | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 2 (2.4) | 3 (4.8) | 0.001 |
| Peru          | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 (1.6) | 1 (1.6) | 0.001 |
| Spain         | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 (1.6) | 1 (1.6) | 0.001 |
| Time from arrival to Spain (median [IQR] (mo)) | 78 (60–100) | 93 (59–103) | 0.40 |
samples were from Bolivian patients (all showed positive titers with IFAT).

Relative sensitivity and relative specificity for all potential S/CO cutoff points for the DBS technique were analyzed by means of an ROC curve (Fig. 2), taking serum sample results as the reference method. Table 3 shows DBS relative sensitivity and relative specificity using the Architect Chagas test in different settings of T. cruzi infection prevalence. Relative sensitivity and relative specificity using DBS were 95.2% and 84.1%, respectively, when a cutoff of ≥1 was considered per the Architect Chagas assay manufacturer’s instructions. When a cutoff of ≥0.88 was considered, sensitivity and specificity were 98.8% and 71.6%, respectively, thus decreasing the proportion of false negatives. In our study, the number of false negatives that occurred with the use of dried blood elutes went from four to only one when the test cutoff was reduced from an S/CO of 1 to an S/CO of ≥0.88.

DISCUSSION

These results show that the use of DBS is a useful, simple, and reliable method for the collection and storage of blood specimens for the detection of IgG against T. cruzi. When a cutoff of ≥0.88 is considered, the relative sensitivity of DBS is high (98.8%), leading to a very low false-negative rate. Although relative specificity is low (71.6%), this should not represent a major problem given that all positive results need to be confirmed with a second technique for a definitive diagnosis of T. cruzi infection.

DBS have been used for the detection of therapeutic antibodies (12, 16), and HIV (17, 18), cytomegalovirus (19), hepatitis (20, 21), and Trypanosoma cruzi (22) IgG.

FIG 1 Dried blood spot (DBS) versus serum T. cruzi IgG values for each patient. The two lines indicate the cutoff values for serum (1.00) and DBS (0.88).

TABLE 2 Detection of IgG antibodies against T. cruzi in DBS versus serum samples using the Architect Chagas assay*

| Sample result | No. positive in serum | No. negative in serum | Total no. |
|---------------|-----------------------|-----------------------|-----------|
| Positive in DBS | 80                    | 10                    | 90        |
| Negative in DBS | 4                     | 53                    | 57        |
| Total         | 84                    | 63                    | 147       |

* DBS, dried blood spots; κ statistic, 0.803 (95% CI, 0.705 to 0.901); Cutoff for DBS, S/CO value of ≥1.00.

FIG 2 ROC curve for DBS results taking serum samples as the reference method. The ROC curve area is 0.972 (95% CI, 0.950 to 0.993).
and herpes simplex antibodies (22). Several in-house and commercial ELISAs have been used to detect IgG against T. cruzi using serum and DBS (23–30). Specimens obtained by minimally invasive methods for the diagnosis of viral, parasitic, or bacterial infections may be preferred where venous blood is difficult to collect/process, such as in community-based or remote settings, in mobile populations, or when sampling is from young children (18). Risks associated with sample transfer/shipping are minimized as DBS cannot be broken in transit and there is no requirement for carriage on dry ice. Handling of potentially infected material is also reduced as the need to centrifuge and separate sera from blood clots is eliminated (31). Filter paper sampling requires overnight elution. However, sample preparation is simple and not time-consuming. Although rapid diagnostic tests also avoid venipuncture, our results reinforce that DBS collection plus application of a highly sensitive and specific ELISA, such as the Architect Chagas test, seems to be an interesting alternative for Chagas disease screening programs. Thus, DBS are a practical alternative to plasma obtained by venipuncture to perform the ELISAs, mainly in situations where rapid sampling and easy specimen storage and transport are required.

Previous studies have shown that optical density (OD) cutoff values for some IgG diagnostic assays differ depending on the sample type (32). Others have reported that IgG antibodies decay in filter paper blood spots after long-term storage, failing to detect IgG in some reactive specimens, which affects the sensitivity of the assay (16). The storage temperature for DBS can also affect reactivity, decreasing the obtained S/CO values even below the reactivity cutoff value (22, 33). Plasma dilution could also alter the OD readings of the assay in positive individuals. In our study, the lower median S/CO values obtained with DBS compared to plasma specimens could have been due to the lower volume of plasma recovered from the 4 dried blood drops as well as the volume of eluent required for the automatic system (minimum 300 μL). These conditions could also explain S/CO values in the gray zone in 3 out of the 4 patients with false-negative values for DBS. Of note, one of these patients showed a borderline S/CO-positive value (1.10) in serum. Further study is needed to determine whether higher S/CO values for IgG-positive individuals may be obtained from DBS by using more dried blood drops or more dots, by eluting the dots in a lower volume, or by reducing the storage time for DBS. Unfortunately, due to a lack of samples, the analyses in these 4 patients could not be repeated.

As regards the 10 false-positive DBS results, in 9 cases the S/CO values were low. The higher S/CO readings for filter paper in some samples could be explained by the presence of interfering proteins from the eluted blood, such as antibodies from other infectious agents or from autoimmune diseases (14). None of these 10 patients had positive serology for leishmaniasis (IFAT), malaria (parasite detection by microscopic examination of blood), or syphilis (ELISA) (data not shown). Absence of T. cruzi antibodies in serum specimens from these 10 subjects was confirmed using a specific IFAT assay. Further studies would be required to ascertain the cause of the higher reactivity in some of the false-positive DBS samples.

To our knowledge, only serum has been used for diagnosis with the FDA-approved Architect Chagas assay (34). Thus, this study is the first reported in Europe in which the use of the Architect Chagas assay was tested with matched DBS-serum samples to detect IgG against T. cruzi in a large number of adults potentially exposed to T. cruzi infection (mainly from areas where this infection is endemic). Most of the immigrants included in our study cohort were from Bolivia, a country with high endemicity for Chagas disease, and 96.4% of them presented T. cruzi-positive serum samples. Further research may include large clinical and field studies involving populations from other countries where Chagas disease is endemic.

While RDTs provide only a qualitative result (either negative or positive), the use of DBS for IgG detection allows optimization of the cutoff value for a given quantitative technique depending on clinical needs and for different epidemiological settings. A high sensitivity is essential for screening programs based on serological tests, as a high proportion of false negatives could be more detrimental than the high number of false positives. In this scenario a false-positive result would be less of a problem given that two different serological tests are required for diagnosis of T. cruzi infection. Thus, all positive results (either true or false) should be confirmed. The reported sensitivity (from 99.85% to 100%) and specificity (from 96.66% to 99.99%) of the Architect assay (13, 34) reinforce its utility as a single screening assay to determine T. cruzi infection status, and confirmation would be required only for those specimens in the gray zone (13).

In summary, using DBS plus the Architect Chagas assay may be an easy and reliable method for the detection of IgG against T. cruzi, with lower false-negative rates than some rapid diagnostic tests. This method would be efficacious and practical for screening at-risk migrants for T. cruzi infection in nonclinical settings and for large epidemiologic field studies in countries where this disease is endemic and where blood sampling by venipuncture may not be feasible. The problem of lower relative sensitivity of DBS compared to the reference method in serum using the Architect system may be overcome by lowering the chemiluminescence signal threshold. These data suggest that before DBS specimens are used for detection of immunoglobulin against any infec-

### Table 3: Potential diagnostic performance of DBS for T. cruzi infection in different scenarios according to published infection rates

| Scenario | Cutoff value (%) | Prevalence (%) | Relative sensitivity (%) | Relative specificity (%) | PPV (%) | NPV (%) | FPR (%) | FNR (%) |
|----------|-----------------|----------------|-------------------------|-------------------------|--------|--------|--------|--------|
| Study sample | 57.1 | 1.0 | 95.2 | 84.1 | 88.9 | 93.0 | 11.1 | 7.0 |
| Study sample | 57.1 | 0.88 | 98.8 | 71.6 | 82.2 | 97.8 | 17.8 | 2.2 |
| Pregnant woman screening (35) | 3.4 | 98.8 | 71.6 | 10.91 | 99.94 | 89.09 | 0.06 |
| Immigrant screening (36) | 4.2 | 98.8 | 71.6 | 13.23 | 99.93 | 86.77 | 0.07 |
| Targeting program (11) | 15.9 | 98.8 | 71.6 | 39.68 | 99.68 | 60.32 | 0.32 |
| Targeting program (37) | 23.6 | 98.8 | 71.6 | 51.80 | 99.48 | 48.20 | 0.52 |
| Immigrant screening (15) | 31.0 | 98.8 | 71.6 | 61.00 | 99.25 | 39.00 | 0.75 |

**Notes:** DBS, dried blood spots; FNR, false-negative rate; FPR, false-positive rate; NPV, negative predictive value; PPV, positive predictive value. Values are given as median S/CO. When S/CO values were low, the higher S/CO readings for filter paper in some samples could be explained by the presence of interfering proteins from the eluted blood, such as antibodies from other infectious diseases and other parasitic infections. The higher S/CO readings for filter paper in some samples could be explained by the presence of interfering proteins from the eluted blood, such as antibodies from other infectious diseases and other parasitic infections.
tion, a previous evaluation of paired DBS-serum specimens would be desirable to optimize the cutoff values of the diagnostic technique which will be used.

ACKNOWLEDGMENTS

This project was carried out with support provided by the Red de Investigación de Centros de Enfermedades Tropicales (RICET) (RED; RD06/0021/0020). This study is included in the “Subprograma de Emigración y Salud from Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública, CIBERESP (Spain).”

Reagents for DBS testing with the Architect Chagas assay were kindly provided by Abbott Laboratories.

REFERENCES

1. Rassi A, Jr, Rassi A, Marin-Neto JA. 2010. Chagas disease. Lancet 375: 1388–1402.
2. Perez-Molina JA, Norman F, Lopez-Velez R. 2012. Chagas disease in non-endemic countries: epidemiology, clinical presentation and treatment. Curr. Infect. Dis. Rep. 14:263–274.
3. Organización Panamericana de la salud (OPS/OMS). 2006. Estimación cuantitativa de la Enfermedad de Chagas en las Américas. OPS/HDM/CD/425-06. Montevideo, Uruguay.
4. Oliveira I, Torrico F, Munoz J, Gascon J. 2010. Congenital transmission of Chagas disease: a clinical approach. Expert Rev. Anti Infect. Ther. 6:945–956.
5. WHO. 2010. Control and prevention of Chagas disease in Europe: report of a WHO Informal Consultation (jointly organized by WHO headquarters and the WHO Regional Office for Europe), Geneva, Switzerland, 17–18 December 2009. WHO, Geneva, Switzerland. http://www.fao.org/ar/1/1comites/chagas/Chagas_WHO_Report_16_06_10.pdf.
6. Basile L, Iansii J, Carlier Y, Salamanca D, Anheben A, Bartoloni A, Seixas J, Van Gool T, Canavate C, Flores-Chavez M, Jackson Y, Chiodini P, Albajar-Vinas P. 2011. Chagas disease in European countries: the challenge of a surveillance system. Euro Surveill. 16 pii:19968. www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19968.
7. Flores-Chavez M, De Fuentes I, Garate T, Cañavate C. 2007. Diagnóstico de laboratorio de la enfermedad de Chagas importada. Enferm. Infect. Microbiol. Clin. 25:Suppl 3:29–37.
8. Brasil PE, De Castro L, Hasslocher-Moreno AM, Sangenis LH, Braga JU. 2010. ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis. BMC Infect. Dis. 10:1337.
9. Chappuis F, Mauris A, Holst M, Albajar-Vinas P, Jannin J, Luquetti AO, Jackson Y. 2010. Validation of a rapid immunochromatographic assay for diagnosis of Trypanosoma cruzi infection among Latin-American Migrants in Geneva, Switzerland. J. Clin. Microbiol. 48:2948–2952.
10. Lopez-Chejade P, Roca C, Posada E, Pinazo MJ, Gascon J, Portus M. 2010. Utility of an immunochromatographic test for Chagas disease screening in primary healthcare. Enferm. Infect. Microbiol. Clin. 28:169–171. (In Spanish.)
11. Navarro M, Perez-Ayala A, Guionnet A, Perez-Molina JA, Navaza B, Estève L, Norman F, Flores-Chavez M, Lopez-Velez R. 2011. Targeted screening and health education for Chagas disease tailored to at-risk migrants in Spain, 2007 to 2010. Euro Surveill. 16 pii:19973. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19973.
12. Prince Pj, Matsuda KC, Retter M, Scott G. 2010. Assessment of DBS technology for the detection of therapeutic antibodies. Bioanalysis 2:149–160.
13. Praast G, Herzogenthal J, Bernhardt S, Christ H, Sicking M. 2011. Evaluation of the Abbott ARCHITECT Chagas prototype assay. Diagn. Microbiol. Infect. Dis. 69:74–81.
14. Flores-Chavez M, Cruz I, Rodríguez M, Nieto J, Franco E, Garate T, Cañavate C. 2010. Comparison of conventional and non-conventional serological tests for the diagnosis of imported Chagas disease in Spain. Enferm. Infect. Microbiol. Clin. 28:284–293. (In Spanish.)
15. Perez-Ayala A, Perez-Molina JA, Norman F, Navarro M, Monge-Maillio B, Díaz-Menendez M, Peris-García J, Flores M, Canavate C, Lopez-Velez R. 2011. Chagas disease in Latin American migrants: a Spanish challenge. Clin. Microbiol. Infect. 17:1108–1113.
16. Rodriguez-Perez MA, Danis-Lozano R, Rodríguez MH, Bradley JE. 1999. Application of an enzyme-linked immunosorbent assay to detect antibodies to Onchocerca volvulus on filter-paper blood spots: effect of storage and temperature on antibody decay. Trans. R. Soc. Trop. Med. Hyg. 93:523–524.
17. Castro AG, Borges LG, Souza Rda S, Grudzinski M, D’Azevedo PA. 2008. Evaluation of the human immunodeficiency virus type 1 and 2 antibodies detection in dried whole blood spots (DBS) samples. Rev. Inst. Med. Trop. Sao Paulo 50:151–156.
18. De Mulder M, Holguin A. 2013. Dried blood spots for monitoring HIV infection in Public Health Programs in developing countries. Enferm. Infect. Microbiol. Clin. 31:100–107. (In Spanish.)
19. Dowd JB, Aiello AE, Chuy L, Huang YY, McDade TW. 2011. Cytomegalovirus antibodies in dried blood spots: a minimally invasive method for assessing stress, immune function, and aging. Immun. Ageing 8:3.
20. Hope YD, Hick BM, Ngui SL, Jones S, Telfer ML, Bizzarri M, Ncobe F, Parry JVI. 2011. Measuring the incidence, prevalence and genetic relatedness of hepatitis C infections among a community recruited sample of injecting drug users, using dried blood spots. J. Viral Hepat. 18:262–270.
21. Mélago JC, Pinto MA, Rocha AM, Freire M, Gaspar LP, Lima SM, Cruz OG, Vital CL. 2011. The use of dried blood spots for assessing antibody response to hepatitis A virus after natural infection and vaccination. J. Med. Virol. 83:208–217.
22. Hogrefe WR, Ernst C, Su X. 2002. Efficiency of reconstitution of immunoglobulin g from blood specimens dried on filter paper and utility in herpes simplex virus type-specific serology screening. Clin. Diagn. Lab. Immunol. 9:1338–1342.
23. Zicker F, Smith PG, Luquetti AO, Oliveira OS. 1990. Mass screening for Trypanosoma cruzi infections using the immunofluorescence, ELISA and haemagglutination tests on dried blood eluates and on blood eluates from filter-paper. Bull. World Health Organ. 68:465–471.
24. Contreras MC, Salinas P, Sandoval L, Solís F, Rojas A. 1992. Usefulness of the ELISA-IgG test in sera and filter paper blood eluates in the Chagas disease immunodiagnosis. Bol. Chil. ParatIALIZ. 47:76–81. (In Spanish.)
25. Palacios X, Belli A, Espino AM. 2000. Detection of antibodies against Trypanosoma cruzi in Somoto, Nicaragua, using indirect ELISA and J1F on blood samples on filter paper. Rev. Panam. Salud Publica 8:411–417. (In Spanish.)
26. Neto EC, Rubin R, Schulte J, Giugliani R. 2004. Newborn screening for congenital infectious diseases. Emerg. Infect. Dis. 10:1068–1073.
27. Segura EL, Escobar-Mesa A; Grupo de Estudio sobre la Enfermedad de Chagas. 2005. Epidemiology of Chagas disease in the state of Veracruz, Mexico. J. Am. Coll. Cardiol. 45:1026–1031.
28. Escríba JM, Ponce E, Romero Ade D, Viñas PA, Marchiol A, Bassets G, Palma PP, Lima MA, Zúñiga C, Ponce C. 2009. Treatment and seroconversion in a cohort of children suffering from recent chronic Chagas infection in Yoro, Honduras. Mem. Inst. Oswaldo Cruz 104:986–991.
29. Frede AF, Luquetti AO, Prata A, Ferreira AW. 2011. Western blotting method (TESAcruzi) as a supplemental test for confirming the presence of anti-Trypanosoma cruzi antibodies in finger prick blood samples from children aged 0–5 years in Brazil. Acta Trop. 117:10–13.
30. Ramos JM, Ponce Y, Gallegos I, Flores-Chávez M, Cañavate C, Gutiérrez F. 2012. Trypanosoma cruzi infection in Elche (Spain): comparison of the serorelevance in immigrants from Paraguay and Bolivia. Pathog. Glob. Health 106:102–106.
31. Parker SP, Cubitt WD. 1999. The use of the dried blood spot sample in epidemiological studies. J. Clin. Pathol. 52:63–639.
32. Weil GJ, Curtis KC, Fischer PU, Won KY, Lammie PJ, Joseph H, Melrose WD, Brattig NW. 2011. A multicenter evaluation of a new antibody test kit for lymphatic filariasis employing recombinant Brugia malayi antigen Bm-14. Acta Trop. 120(Suppl 1):S19–S22.
33. Joseph HM, Melrose W. 2010. Applicability of the filter paper technique for detection of anti-filarial IgG(4) antibodies using the Bm14 filariasis CELISA. J. Parasitol. Res. pili:594687. doi:10.1155/2010/594687.
34. Bohra-Bendicho MA, Albert-Hernández M, Márquez-Contreras C, Segovia-Hernández M. 2012. ARCHITECT Chagas(®): a new diagnostic tool in Chagas disease. Enferm. Infect. Microbiol. Clin. 30:463–465. (In Spanish.)
35. Muñoz J, Coll O, Juncosa T, Verges M, del Pino M, Fumado V, Bosch J, Posada EJ, Hernandez S, Fisa R, Boguna JM, Gallego M, Sanz S, Portus M, Gascon J. 2009. Prevalence and vertical transmission of
Trypanosoma cruzi infection among pregnant Latin American women attending 2 maternity clinics in Barcelona, Spain. Clin. Infect. Dis. 48:1736–1740.

36. Angheben A, Anselmi M, Gobbi F, Marocco S, Monteiro G, Buonfrate D, Tais S, Talamo M, Zavarise G, Strohmeyer M, Bartalesi F, Mantella A, Di Tommaso M, Aiello K, Veneruso G, Graziani G, Ferrari M, Spreafico I, Bonifácio E, Gaiera G, Lanzafame M, Mascarello M, Cancrini G, Albajar-Vinas P, Bisoffi Z, Bartoloni A. 2011. Chagas disease in Italy: breaking an epidemiological silence. Euro Surveill. 16:pii=19969. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19969.

37. Lescure FX, Paris L, Elghouzzi MH, Le Loup G, Develoux M, Touafek F, Mazier D, Pialoux G. 2009. Experience of targeted screening of Chagas disease in Île-de-France. Bull. Soc. Pathol. Exot. 102:295–299. (In French.)