

# Use of an Immunochromatographic Dipstick Test for Rapid Detection of *Trypanosoma cruzi* in Sera from Animal Reservoir Hosts

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**We evaluated an immunochromatographic dipstick test to detect *Trypanosoma cruzi* in canine serum samples from areas of endemicity ( $n = 141$ ) and nonendemicity ( $n = 28$ ) for Chagas' disease with known serological and xenodiagnostic test results. The dipstick test had a specificity of at least 94% and a sensitivity of at least 96%. The dipstick tested could become the first choice for screening purposes in disease surveillance or intervention programs.**

Chagas' disease is the most important parasitic disease in the Americas, where it causes an estimated 0.67 million disability-adjusted life years; 18 million people are currently infected, with up to 100 million at risk of the disease (17). It is caused by *Trypanosoma cruzi*, a protozoan parasite transmitted to the host by blood-sucking triatomine bugs (12).

Although there are several methods for the diagnosis of *T. cruzi* infection, none are ideal for epidemiological surveys, which require mass screening of samples. While serological tests (i.e., enzyme-linked immunosorbent assay [ELISA], immunofluorescence antibody test [IFAT], or indirect hemagglutination test [IHAT]) are comparatively easy to use and sensitive, their specificities vary (1, 8, 15). Molecular tests (i.e., PCR) are very specific, but they lack sensitivity and require technological expertise and specialized, expensive laboratory equipment (11). Hemoculture and xenodiagnosis are the current "gold standards" for the parasitological diagnosis of *T. cruzi* infection (11). Although these techniques are specific, in the chronic phase of infection their sensitivities are highly variable (e.g., 0 to 50% [4]); they also are labor-intensive and time-consuming (e.g., because of the necessity to mass rear bugs for xenodiagnosis). Thus, a rapid, sensitive, and specific diagnostic test for mass screening surveys and intervention campaigns would be extremely valuable: the results could be read immediately and control measures could be implemented in situ.

Immunochromatographic dipstick tests have been developed for a range of tropical diseases, including malaria (16), leishmaniasis (14), and schistosomiasis (2); until recently (9, 10), none was available for Chagas' disease.

Because cats and especially dogs are important reservoir hosts of *T. cruzi* (6), they could be targeted in a Chagas' disease control strategy (e.g., as sentinels in a surveillance program or by culling or collaring [13]) if the infected animals can be identified accurately. In most areas where Chagas' disease is endemic, the diagnosis of *T. cruzi* infection in humans or domestic animals relies on ELISA and IFAT (8, 15).

For the first time, we evaluated the sensitivity and specificity of a commercially available immunochromatographic dipstick test for the detection of *T. cruzi* infection in canine serum samples with known serological and xenodiagnostic test results collected in areas where Chagas' disease is endemic and non-endemic.

**Test samples.** We used archived samples stored at  $-20^{\circ}\text{C}$  that had been collected between 2000 and 2003 from dogs during extensive epidemiological studies of Chagas' disease in (i) Amamá and neighboring villages in Santiago del Estero Province of Argentina (3) and (ii) in a delimited area between the Teuco and the Bermejito Rivers in Chaco Province, northern Argentina. Xenodiagnosis had also been undertaken for some of these dogs ( $n = 29$ ) (11); i.e., the samples had a known parasitological result. We tested samples by at least two of the following standardized methods (3, 8): ELISA (cutoff value [optical density reading], 0.2;  $n = 141$ ), IFAT (cutoff value [titer], 1:16;  $n = 141$ ), and IHAT (cutoff value [titer], 1:16;  $n = 69$ ). Additionally, serum samples ( $n = 28$ ) from dogs residing and attending a veterinary hospital (Instituto "Luis Pasteur") in Buenos Aires City were tested by both ELISA and IFAT; vector-borne *T. cruzi* infection is not endemic in Buenos Aires. In this study the samples were classified according to the diagnostic protocol guidelines of the Instituto Nacional de Parasitología, where a sample was considered "serologically positive" if it was positive by two or more standard serological tests (1). For convenience, in this study we use the term "serologically discordant" for serum samples that were positive by only one test among ELISA, IFAT, and IHAT and for sera for which the results of the dipstick test and standard serology differed.

**Dipstick test.** The dipstick test (*Trypanosoma cruzi* Detect-Canine; Inbios, Seattle, WA) was carried out according to the manufacturer's instructions. Serum (20  $\mu\text{l}$ ) and the provided chase buffer solution (150 to 200  $\mu\text{l}$ ) were added onto the dipsticks. After 10 min, a red control line and, if the result was positive, a second line appeared on the test field. The test is based on a proprietary gold mix containing multiepitope recombinant antigens ITC-6 and ITC-8.2 derived from different *T. cruzi* antigens, including peptide 2, TcD, TcF, TcLo, and

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TABLE 1. Comparative diagnosis of *T. cruzi* in canine blood samples collected in locations of endemicity in northern Argentina<sup>a</sup>

Dipstick test result	No. of serum samples					
	Serology positive			Serology negative		
	XENO positive	XENO negative	XENO ND	XENO positive	XENO negative	XENO ND
Positive	19 <sup>b</sup>	7	40 <sup>c</sup>	1	1	5 <sup>d</sup>
Negative	0	0	3 <sup>e</sup>	0	1	64

<sup>a</sup> The *Trypanosoma cruzi* Detect-Canine dipstick was used. Twenty-eight samples from dogs residing in Buenos Aires City were negative by all serological tests. Serology, samples were tested by enzyme-linked immunosorbent assay and immunofluorescence antibody test, and a fraction was also tested by the hemagglutination test; XENO, xenodiagnosis; ND, not done.

<sup>b</sup> Includes one seropositive sample that was reactive only by ELISA and IFAT.

<sup>c</sup> Includes three seropositive samples that were reactive only by IFAT and IHAT.

<sup>d</sup> Includes two samples that were reactive only by IFAT and one sample that was reactive only by IHAT.

<sup>e</sup> Includes two seropositive samples that were reactive only by ELISA and IFAT and by IHAT and IFAT, respectively.

SAPA (Syamal Raychaudhuri, Inbios, personal communication).

**Results.** Table 1 summarizes the dipstick, serology, and xenodiagnosis results for field samples. A total of 73/141 (52%), 69/141 (49%), and 20/29 (69%) of the samples tested positive by dipstick, serology, and xenodiagnosis, respectively. There was no statistically significant difference (as determined by the chi-square test for proportions) between the numbers of samples testing positive by the dipstick test or serology.

None of the 28 negative canine control samples from Buenos Aires City were positive by ELISA, IFAT, or the dipstick test. A total of 10/141 (7.1%) samples tested had discordant results between the dipstick test and the standard serological tests (Table 1); one of these samples was from a xenodiagnosis-positive dog correctly detected as positive by the dipstick test but not by standard serology. Of these 10 samples with discordant results, two seropositive samples that tested negative by the dipstick test were reactive only by ELISA and IFAT or by IHAT and IFAT, respectively; and two seronegative samples that tested positive by the dipstick test were reactive only by IFAT or IHAT.

To estimate the sensitivity and the specificity of the dipstick test, the true number of infected dogs must be known; two approaches were used. In approach 1, estimates were based on the results for known negative control field samples (i.e., negative control samples from Buenos Aires City) and positive field samples (i.e., samples from xenodiagnosis-positive dogs) by the standard methodology (5). All negative control samples were negative by the dipstick test (i.e., 100% specificity), and 20 of 20 xenodiagnosis-positive samples were also positive by the dipstick test (i.e., 100% sensitivity). In approach 2, estimates were based on the results for field and control samples negative by at least serology (a few were also negative by xenodiagnosis) and field samples positive by xenodiagnosis and/or serology, respectively. A total of 93 of 99 serology-negative (and, if tested, xenodiagnosis-negative) field and control samples were also negative by the dipstick test (i.e., 94% specificity), and 67 of 70 serology- and/or xenodiagnosis-positive samples were also positive by the dipstick test (i.e., 96% sensitivity).

**Discussion.** To our knowledge this is the first study to use an immunochromatographic dipstick to detect *T. cruzi* infection in reservoir animals and the first study to test this particular brand of dipstick test. Our study indicates that the dipstick tested has a high specificity (>94%) and a high sensitivity (>96%). The dipstick tested had a higher sensitivity than xenodiagnosis (of 27 samples that were serologically and dipstick positive, only 20 [74%] were also xenodiagnosis positive; Fisher's exact test,  $P < 0.01$ ) and a sensitivity comparable to that of standard serology with ELISA and IFAT (8). Recently, another commercial dipstick test (Chagas Stat-Pak; Chembio Diagnostics Inc., Medford, NY) showed a similarly high sensitivity and a similarly high specificity as the dipstick test evaluated here when it was tested with a panel of randomly selected human serum samples (9) as well as 3,400 human samples from blood banks in Central America (10).

We also show, as in similar diagnostic studies that have evaluated PCR (11), that standard serological tests can yield false-negative results, as evidenced by one of two serologically negative samples that tested dipstick positive and that was also positive by the use of xenodiagnosis. Study records indicated that the dog from which the sample was obtained inhabited a house with *T. cruzi*-infected *Triatoma infestans* bugs and was probably an acute case at the initial stage of infection, when standard serology is commonly unresponsive.

We cannot exclude the possibility that the dipstick test would lead to a small proportion of false-negative or false-positive results for serum samples. As similar studies evaluating dipsticks for malaria, leishmaniasis, and schistosomiasis diagnosis have shown, variability in dipstick test performance will depend on factors such as the type of diagnostic antigen and the conjugate used. Commercially available dipstick tests can be highly variable in terms of their sensitivities and specificities. For example, the rates of false-positive results for malaria dipsticks were reportedly as high as 28%, probably due to cross-reactivity to rheumatoid factor (7). For the study dogs residing in Chaco Province and for one dog from Santiago del Estero Province, false-positive results caused by cross-reaction with *Leishmania* spp. could be possible (8).

There are many potential advantages of using dipsticks rather than other diagnostic methods. First, when dipsticks are used, a large number of samples can be processed quickly and with minimum effort. Second, compared to serology, molecular methods, or xenodiagnosis, the technological expertise (i.e., training of personnel) necessary to perform the dipstick test is minimal, as is the requirement for specialized laboratory equipment. Another advantage of dipstick tests is that patients (or, in this case, dog owners) can see the results for themselves, which will contribute to a better working relationship between the individuals in the local communities and the people carrying out the surveys. Third, from an epidemiological point of view, a dipstick test allows intervention strategies to be implemented in situ, such as for serological surveillance; collaring; culling; or identifying (un-)infected animals for parasite isolation, vaccine, or clinical trials. This reduces the need for repeated visits to houses, which are necessary when the currently used serological tests are performed, thereby reducing operational program costs.

Immunochromatographic dipstick tests are comparatively expensive (the dipstick tested here has a retail price of \$2.00),

but considering the findings presented above, a sensitive and specific dipstick test such as the one tested here could prove to be a very cost-effective alternative to the currently available diagnostic tests, especially when it is used in mass screening surveys.

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