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Field validation of a *Leishmania (Leishmania) mexicana* exo-antigens ELISA for diagnosing tegumentary leishmaniasis in regions of *Leishmania (Viannia)* predominance

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ABSTRACT

Background: Several tests are performed to obtain better accuracy when diagnosing American tegumentary leishmaniasis (ATL). It is believed that antigens released via secretion, excretion and metabolism are more specific than are antigens released by the lysis of *Leishmania* parasites. Such antigens are known as exo-antigens (exo-Ag) and are formed from products released by cultured parasites in a way that is similar to that in which they cause infections in hosts.

Objective: We attempted to validate a *Leishmania mexicana* ELISA exo-Ag for ATL diagnosis in Midwestern Brazil.

Methods: A total of 281 patients were included in the study. We analysed pre-treatment blood from 98 ATL patients; out of those, 85.7% and 14.3% had cutaneous and mucosal forms, respectively.

Results: The exo-Ag accuracy was 83.99% (95% CI = 79.24–87.81) with a sensitivity value of 90.82% (95% CI = 83.46–95.09) and an overall specificity value of 80.33% (95% CI = 73.97–85.44). The positive predictive value and negative predictive value were 71.20% (95% CI = 62.72–78.41) and 94.23% (95% CI = 89.40–96.94), respectively. Among healthy controls, exo-Ag had a specificity of 91.25% (95% CI = 83.02–95.70); additionally, the test had

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specificity rates of 66.67% (95% CI = 46.71–82.03) in Chagas disease patients, 60.61% (95% CI = 43.68–75.32) in patients with rheumatic diseases, 76.92% (95% CI = 49.74–91.82) in pemphigus foliaceus patients, 87.50% (95% CI = 52.91–97.76) in leprosy patients, 87.50% (95% CI = 63.98–96.50) in VRDL-positive patients, and 77.78 (95% CI = 45.26–93.68) in deep mycosis patients.

Conclusion: Based on the indicators of validity, we conclude that the results obtained in this study enable the recommendation of the exo-Ag ELISA for ATL diagnosis once it presented a reasonable accuracy compared to classical methods. Cost evaluations are necessary to completely define the role of this technique in large scale.

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Introduction

American tegumentary leishmaniasis (ATL) is an infectious and difficult-to-control disease that is caused by parasites of the genus *Leishmania*. ATL causes considerable socioeconomic losses, as it affects subjects at their most productive ages.[1]

Several complimentary tests are used to diagnose ATL, all of which have limitations. The diagnostic tests include parasite detection techniques (e.g., direct examination, cultures, histopathological examinations and PCR) and immunodiagnostic techniques (e.g., cellular immune response detection, including the Montenegro skin test (MST), antibody detection, including the fluorescent antibody test (IFAT), ELISA, and immunocomplex antigen detection).[2,3] Serological tests have been given considerable importance in epidemiological investigations. However, for diagnostic purposes, those methods can be associated with a relatively high proportion of false-positive results.

ELISAs have a great advantage among other serological tests because of their higher specificity. In addition to quickly generated results, ELISAs are also relatively low-cost tests.[4,5] The antigens most commonly used in leishmaniasis diagnostic immunoenzymatic assays are obtained from intact parasites. The sensitivities of these assays fluctuate between 85% and 100% for crude antigens.[6] Crude antigens from *Leishmania (Leishmania) amazonensis* and *Leishmania (Viannia) braziliensis*, when separated by electrophoresis and subjected to Western blotting, can be recognized by sera from patients with Chagas disease.[7] This phenomenon is believed to occur because of interactions with *Trypanosoma* and *Leishmania* common antigens.[8]

On the other hand, it is believed that antigens released via secretion, excretion and metabolism are more specific than are antigens released by the lysis of *Leishmania* parasites.[9,10] Such antigens are known as exo-antigens (exo-Ag) and are formed from products released by cultured parasites in a way that is similar to that in which they cause infections in hosts.[11] An ELISA with *Leishmania (Leishmania) mexicana (L. mexicana)* promastigote antigens, used to detect IgM and IgG antibodies in sera from Brazilian *Leishmania*-infected patients, had a sensitivity of 92.3% and was reactive when tested in 10 patients with ATL via Western blotting.[12]

ELISA with an exo-Ag from *Leishmania* parasites cultured in protein-free medium is thought to be an improved method with increased accuracy; therefore, we intended to validate this technique for ATL diagnosis in Midwestern Brazil.

Materials and methods

This study was conducted from August 2007 to July 2010 at the Hospital Universitário de Brasília, Brasília, Brazil, a reference centre for ATL in Midwestern Brazil. Ninety-eight consecutive patients with cutaneous (CL) and mucocutaneous (MCL) disease forms, who were specific treatment naive during the previous six months, were included in a cross-sectional study for diagnostic test accuracy.

ATL diagnosis was performed according to a previously validated composite reference standard formed by clinical and laboratory criteria (direct examination, culture, histopathological examination, PCR from lesion fragments, MST and IFAT).[13] *Leishmania* subgenus identification was performed at the Laboratory of Dermatology, Hospital das Clínicas, University of São Paulo, Ribeirão Preto, Brazil, using restriction fragment length polymorphism (RFLP) as described elsewhere.[13]

Additionally, the study included a convenience sample of 80 healthy patients with no epidemiological history of leishmaniasis, 24 patients with Chagas disease, 13 patients with American pemphigus foliaceus, 8 patients with lepromatous leprosy, 9 patients with deep mycosis, 16 VRDL-positive patients at a minimum dilution of 1:8, and 33 patients with rheumatic disease who were positive for at least one rheumatic marker test, including anti-streptolysin O (ASO), C-reactive protein (CRP), and rheumatoid factor (RF).

Sampling

Two millilitres of patient serum, obtained through venipuncture, was used. After clot retraction, the samples were centrifuged and stored in labelled tubes at -20° C until the ELISA exo-Ag analyses were performed.

L. mexicana exo-antigen ELISA

A manufactured kit was used for the ELISA reactions. The kit, developed by the Cellabs laboratory (Brookvale, Australia), contained the following components: one ELISA plate with 12 detachable strips, each of which contained eight wells pre-coated with *Leishmania* exo-antigens; one vial of washing buffer (PBS-Tween, 20× concentrate); one vial of

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