

NOTES

Rapid Immunochromatographic Strip Test for Detection of Anti-K39 Immunoglobulin G Antibodies for Diagnosis of Visceral Leishmaniasis[∇]

Ryan J. Welch,^{1*} Brian L. Anderson,¹ and Christine M. Litwin^{1,2}

Associated Regional and University Pathologists Institute for Clinical and Experimental Pathology¹ and Department of Pathology, Pediatrics and Medicine,² University of Utah School of Medicine, Salt Lake City, Utah

Received 16 May 2008/Returned for modification 5 June 2008/Accepted 1 July 2008

InBios International has developed an immunochromatographic rapid strip for the detection of visceral leishmaniasis that requires minimal equipment and only a small amount of blood to run a test. We compared the InBios rapid strip test with the CDC immunofluorescent antibody assay, and the agreement, sensitivity, and specificity were 98%, 90%, and 100%, respectively.

Visceral leishmaniasis (VL) is the result of infection by the *Leishmania donovani* species complex (including *L. donovani*, *Leishmania chagasi*, and *Leishmania infantum*) and *Leishmania tropica* (11, 15). The recommended method for diagnosis has been microscopic determination of parasites from bone marrow, splenic, or lymphatic tissue biopsy specimens (7); however, these tests are invasive as well as difficult to perform in rural areas, where VL may be endemic and carry a high risk of complication (6, 17). Recently, real-time PCR has been utilized to diagnose *Leishmania* infection and appears promising; nevertheless, the ability to perform the assay becomes prohibitive in many regions of endemicity with limited medical resources (10, 12, 16). It has therefore been a goal in VL testing to produce a rapid, noninvasive technique for diagnosis that can be used in the field (5, 7, 13). InBios International, Inc. (Seattle, WA), has developed such a test that has been approved by the Food and Drug Administration.

The InBios Kalazar Detect rapid test utilizes the recombinant *L. chagasi* antigen rK39, which is a 39-amino-acid repeat section in the 230-kDa LcKin protein (1). It has previously been reported that *L. chagasi*, *L. donovani*, and *L. infantum* all contain the gene encoding the LcKin protein (1). A membrane strip which also contains a conjugate dye region is coated with this protein. Through capillary action, the patient serum will react with the dye and antigen to quickly indicate the presence of anti-rK39 immunoglobulin G (IgG) in a patient sample.

In this study, we determined the efficacy of the InBios VL test by comparing results of the InBios test with results from the test used by the Centers for Disease Control and Prevention (CDC; Atlanta, GA).

Human sera. This study was approved by the Institutional Review Board of the University of Utah (IRB 7275). Serum samples were divided into two categories: samples that were

obtained from patients proven to have leishmaniasis by the CDC, and samples that were sent to ARUP Laboratories (Salt Lake City, UT) for leishmaniasis serological testing. Sixteen samples that had been collected from patients treated by the CDC for leishmaniasis and pathologically confirmed by PCR, culture, or direct detection at the CDC for leishmaniasis were de-identified and stored at 2 to 8°C. Seventy-eight serum samples that were originally collected between 2007 and 2008 and sent to ARUP Laboratories for leishmaniasis testing were de-identified and stored at 2 to 8°C.

InBios Kalazar Detect rapid test. All 94 samples were tested for anti-rK39 IgG antibodies using the InBios Kalazar Detect rapid test (Seattle, WA) according to the manufacturer's protocol. Patient serum was placed on the assigned location on the immunochromatographic strip, and the strip was placed in a tube containing InBios chase buffer solution. Following a 10-minute incubation at room temperature, the strips were analyzed. The presence of a visible band in the designated location indicated anti-rK39 IgG in the sample and therefore a VL-positive patient.

CDC testing. All 94 samples were assayed at the CDC according to the CDC immunofluorescent antibody (IFA) test protocol for anti-*Leishmania donovani* antibodies.

Parasite serology. Two samples that tested negative on the InBios rapid strip test and positive on the CDC IFA were tested for IgG antibodies against other infectious parasites, including *Toxocara* spp. (r-Biopharm, Darmstadt, Germany), *Strongyloides stercoralis* (IVD Research, Inc., Carlsbad, CA), *Taenia solium* (IVD Research, Inc.), *Trypanosoma cruzi* (IVD Research, Inc., and InBios International, Inc.), *Echinococcus* spp. (Thermo Fisher Scientific, Lenexa, KS), *Entamoeba histolytica* (IVD Research, Inc.), and *Trichinella spiralis* (IVD Research, Inc.). All assays were run according to the manufacturers' protocols.

Statistical analysis. To determine the overall agreement, clinical sensitivity, clinical specificity, and 95% confidence intervals (CI) for sensitivity and specificity, two-by-two contingency table analysis was used (4). Results of the InBios test were compared to the CDC results, and any samples that

* Corresponding author. Mailing address: ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108. Phone: (801) 583-2787, ext. 3540. Fax: (801) 584-5109. E-mail: ryan.welch@aruplab.com.

[∇] Published ahead of print on 16 July 2008.

disagreed were repeated on the InBios test to ensure accurate results. Agreement, sensitivity, and specificity were 98%, 90% (95% CI, 78 to 90%), and 100% (95% CI, 97 to 100%), respectively.

The 16 CDC-confirmed positive serum samples all tested positive on the InBios VL test. Of the 78 samples originally sent to ARUP Laboratories for leishmaniasis testing, 77 tested negative on the InBios test and 1 tested positive. All 78 samples were then sent to the CDC for IFA testing, and three samples had positive titer values, including the sample that tested positive on the InBios VL test. Of the two samples that were positive on the CDC IFA assay but negative on the InBios VL test, one sample tested positive for antibodies against *Toxocara* spp. Although it has been determined that cross-reactivity exists between leishmaniasis serology and other parasite serology, especially with *Trypanosoma* spp. (8), it cannot be conclusively proven that the positive results of the CDC IFA test are due to *Toxocara* spp. The IgG test for *Trypanosoma cruzi* would be the most likely indicator of cross-reactivity, and as both samples were negative for antibodies against *T. cruzi*, this can be ruled out. Further testing for antibodies against *Trypanosoma brucei* would be needed to fully rule out *Trypanosoma* cross-reactivity, but this testing was not available.

Several studies in India and Brazil have concluded that the InBios rapid strip test for diagnosis of VL is highly sensitive and specific (2, 9, 14), and the U.S. Army currently uses the InBios rapid strip on suspected VL in returning soldiers and on the local Iraq population (3, 6, 15). The results of these studies and our own demonstrate that the InBios rapid strip has a high sensitivity and specificity for VL detection in multiple countries on different continents, where the species responsible for VL may differ (7).

This work was supported by the ARUP Institute for Clinical and Experimental Pathology.

We give special thanks to InBios International, Inc., for providing reagents, Frank Steurer from the CDC in Atlanta, GA, for providing confirmatory testing and positive serum samples, and Colonel Peter J. Weina from the Walter Reed Army Institute of Research for providing information on current U.S. Army testing methodologies.

REFERENCES

1. Burns, J. M., Jr., W. G. Shreffler, D. R. Benson, H. W. Ghalib, R. Badaro, and S. G. Reed. 1993. Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. *Proc. Natl. Acad. Sci. USA* **90**:775–779.
2. Carvalho, S. F., E. M. Lemos, R. Corey, and R. Dietze. 2003. Performance of recombinant K39 antigen in the diagnosis of Brazilian visceral leishmaniasis. *Am. J. Trop. Med. Hyg.* **68**:321–324.
3. Centers for Disease Control and Prevention. 2004. Two cases of visceral leishmaniasis in U.S. military personnel—Afghanistan, 2002–2004. *MMWR Morb. Mortal. Wkly. Rep.* **53**:265–268.
4. Fleiss, J. L. 1981. *Statistical methods for rates and proportions*, 2nd ed. John Wiley & Sons, New York, NY.
5. Guerin, P. J., P. Olliaro, S. Sundar, M. Boelaert, S. L. Croft, P. Desjeux, M. K. Wasunna, and A. D. Bryceson. 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect. Dis.* **2**:494–501.
6. Halsey, E. S., L. M. Bryce, G. W. Wortmann, P. J. Weina, J. R. Ryan, and C. C. DeWitt. 2004. Visceral leishmaniasis in a soldier returning from Operation Enduring Freedom. *Mil. Med.* **169**:699–701.
7. Herwaldt, B. L. 1999. Leishmaniasis. *Lancet* **354**:1191–1199.
8. Kar, K. 1995. Serodiagnosis of leishmaniasis. *Crit. Rev. Microbiol.* **21**:123–152.
9. Mathur, P., J. Samantaray, and N. K. Chauhan. 2005. Evaluation of a rapid immunochromatographic test for diagnosis of kala-azar & post kala-azar dermal leishmaniasis at a tertiary care centre of north India. *Indian J. Med. Res.* **122**:485–490.
10. Nuzum, E., F. White III, C. Thakur, R. Dietze, J. Wages, M. Grogg, and J. Berman. 1995. Diagnosis of symptomatic visceral leishmaniasis by use of the polymerase chain reaction on patient blood. *J. Infect. Dis.* **171**:751–754.
11. Pearson, R., A. De Queiroz Souza, and S. Jeronimo. 2000. *Leishmania* species: visceral (kala-azar), cutaneous, and mucosal leishmaniasis. In G. L. Mandell, R. G. Douglas, J. E. Bennett, and R. Dolin (ed.), *Principles and practice of infectious diseases*, 5th ed. Churchill Livingstone, Philadelphia, PA.
12. Seaman, J., A. J. Mercer, H. E. Sondorp, and B. L. Herwaldt. 1996. Epidemic visceral leishmaniasis in southern Sudan: treatment of severely debilitated patients under wartime conditions and with limited resources. *Ann. Intern. Med.* **124**:664–672.
13. Sundar, S., S. G. Reed, V. P. Singh, P. C. Kumar, and H. W. Murray. 1998. Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* **351**:563–565.
14. Sundar, S., M. Sahu, H. Mehta, A. Gupta, U. Kohli, M. Rai, J. D. Berman, and H. W. Murray. 2002. Noninvasive management of Indian visceral leishmaniasis: clinical application of diagnosis by K39 antigen strip testing at a kala-azar referral unit. *Clin. Infect. Dis.* **35**:581–586.
15. Weina, P. J., R. C. Neafie, G. Wortmann, M. Polhemus, and N. E. Aronson. 2004. Old World leishmaniasis: an emerging infection among deployed US military and civilian workers. *Clin. Infect. Dis.* **39**:1674–1680.
16. Wortmann, G., L. Hochberg, H. H. Houng, C. Sweeney, M. Zapor, N. Aronson, P. Weina, and C. F. Ockenhouse. 2005. Rapid identification of *Leishmania* complexes by a real-time PCR assay. *Am. J. Trop. Med. Hyg.* **73**:999–1004.
17. Zijlstra, E. E., M. S. Ali, A. M. el-Hassan, I. A. el-Toum, M. Satti, H. W. Ghalib, and P. A. Kager. 1992. Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis. *Trans. R. Soc. Trop. Med. Hyg.* **86**:505–507.