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Cloning, expression and purification of *L. Donovani* specific antigen for serodiagnosis of visceral leishmaniasis

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Microscopic observation of amastigotes in splenic or bone marrow biopsies and serology including rK39 dipstick test are tools commonly used for diagnosis of visceral leishmaniasis (VL). However, bone marrow and splenic aspiration are painful and risky procedures and serology with rK39 dipstick can yield false positive responses in 20-32% of endemic healthy individuals. Identification of additional *Leishmania donovani* antigens could increase the specificity of noninvasive serologic testing for active VL.

Therefore, we screened promastigotes soluble proteins using western blotting with a series of serum specimens from patients with acute VL. Western blots revealed a protein of molecular weight 70kDa (BHUP1), recognized by sera from VL patients but not healthy controls. Mass spectrometry of the gel-purified protein revealed the antigen as *L. donovani* HSP70. The full length *hsp70* gene of 1959 nucleotides was determined, cloned and expressed as a His-tagged fusion protein, purified, and retested.

Antibody against this protein were detected in more than 96% of serum samples from patient with VL but not detected in sera from the endemic and non endemic control persons. Cross-reactive responses of sera from subjects with different disease like malaria and tuberculosis revealed the BHUP1 antigen test is highly sensitive for VL, but specificity was too low to differentiate from other infectious diseases

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