SPECIES-SPECIFIC *Leishmania* DNA DETECTION USING THE LAMP-BASED DIAGNOSTIC TOOL LeishID

ALESSANDRA MARA DE SOUSA[1]; ELLEN GONÇALVES DE OLIVEIRA[1];RAPHAEL FRANÇA OLIVEIRA[1]; RAFAEL LUIZ DA SILVA NEVES[1]; EDUARDO MARTINEZ[2]; CÉLIA MARIA FERREIRA GONTIJO[3]; RODRIGO PEDRO SOARES[1]; VINICIUS MARACAJÁ COUTINHO[2]; RUBENS L. DO MONTE-NETO[1]

[1]GRUPO DE PESQUISAS EM BIOTECNOLOGIA APLICADA AO ESTUDO DE PATÓGENOS (BAP) - INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ - IRR/FIOCRUZ MINAS, BELO HORIZONTE, MG, BRASIL; [2]CENTRO DE MODELAMIENTO MOLECULAR BIOFISICA Y BIOINFORMATICA - CMB, FACULTAD DE CIENCIAS QUIMICAS Y FARMACÉUTICAS, UNIVERSIDAD DE CHILE, SANTIAGO, CHILE; [3]GRUPO DE ESTUDOS EM LEISHMANIOSE - INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ - IRR/FIOCRUZ MINAS, BELO HORIZONTE, MG, BRASIL.

Abstract

Sensitive, reliable, and rapid diagnostic tools are crucial to tackle leishmaniases. Recent reports have revealed *Leishmania* species associated with dermotropic forms causing visceral leishmaniases, and *vice versa*. This poses a problem for epidemiology and a challenge to better understand the dynamics of the pathophysiology of leishmaniases. Although several molecular methods detect *Leishmania*, most do not identify it at the species level. The loop-mediated isothermal DNA amplification (LAMP) technique stands out for its ease of execution, and high sensitivity, without the need for a thermal cycler. In this context, here we developed LeishID, a LAMP-based diagnostic method to specifically identify *L. infantum*, *L. braziliensis*, and *L. amazonensis.* We selected species-specific targets from 26 publicly available *Leishmania* genomes, covering at least 16 different species. The accessory genome was filtered to obtain unique sequences for each species. LAMP primers were designed and the displacement probe technique was applied for multiplex assay purposes, using fluorophore-labeled loop primers. For the LAMP assay, we used the WarmStart Colorimetric LAMP mix (NEB). Depending on the primers used, 15 min of incubation is enough to detect *Leishmania* DNA, although 40 min offers greater sensitivity, with detection limits ranging from 0.01 fg to 1 pg. The use of specific primers ensured accurate identification of the analyzed species, without cross-reactions. Clinical validation was performed on 47 spleen biopsy specimens obtained from dogs with canine leishmaniasis and on 43 clinical specimens derived from skin lesions of patients with cutaneous leishmaniasis from Minas Gerais, Brazil. LeishID was able to identify *L. infantum* and *L. braziliensis*, in spleen and skin lesions, respectively, with a sensitivity of 97.9% and a specificity of 100%. Thus, LeishID is rapid, specific, and sensitive in differentiating *Leishmania* species at clinically relevant concentrations.

Keywords: *Leishmania*; LAMP; diagnostics
Financial Support: Fapemig (RED-0032-22; RED-00196-23), CAPES, CNPq (312353/2023-5), Fiocruz