DEVELOPMENT OF MOLECULAR TEST BASED ON UNUSUAL ISOTHERMAL MULTIMERIZATION AND AMPLIFICATION (UIMA) FOR THE DIAGNOSIS OF TEGUMENTARY LEISHMANIASIS (TL)

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Tests based on isothermal amplification of nucleic acids have the potential to facilitate the decentralization of molecular diagnosis of tegumentary leishmaniasis (TL). Previous studies have shown that Bst DNA polymerase enzymes with reverse transcription activity can be used to amplify DNA by multimerization (UIMA), using a single primer or primers for different genomic regions. The aim of this study was to evaluate the use of sense (150) and antisense (152) primers for *Leishmania* kDNA in the UIMA technique for the diagnosis of TL. The selected primers were individually evaluated using different concentrations of the enzyme 2.0 Bst DNA polymerase WarmStart (4 to 8 U), reaction temperature (63ºC) and reaction time (30 to 180 min) in a water bath. The results were visualized “with the naked eye” using Sybr Green I 10,000X/DMSO. Furthermore, 4 µL of the UIMA products were visualized in a 6% polyacrylamide gel (6% PAGE) stained with silver. After standardization, the detection limit (1 ng to 1 fg of DNA) of the kDNA-UIMA assay was evaluated in comparison with the kDNA-qPCR assay. *Leishmania* DNA detection was only possible using primer 152 (1.5 µM) and the reagents at the following final concentrations: 2.0 Bst DNA polymerase WarmStart (8U), Bst enzyme buffer (1X), MgSO4 (6 mM); dNTPS (2 mM each dNTP). The 6% PAGE analysis revealed the presence of amplicons only in the positive controls for 120 and 180 minutes. The results obtained by visual interpretation were the same as those obtained by 6% PAGE analysis. The detection limit of kDNA-UIMA assay was 100 pg, lower than that of kDNA-qPCR (1 fg). Although the detection limit of kDNA-UIMA was lower than that of kDNA-qPCR, other primer sets will be evaluated, which may also be evaluated in association with primer 152, to increase the detection limit of the UIMA for the diagnosis of TL. The parameters of analytical specificity and clinical accuracy will also be evaluated.

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