IDENTIFYING POSSIBLE MISMATCH POINTS IN PRIMERS REGIONS OF HSP70-LAMP ASSAY FOR THE DIAGNOSIS OF TEGUMENTARY LEISHMANIASIS

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Nucleic acid tests (NATs) are fast and highly accurate tools for diagnosing infectious diseases. Loop-mediated Isothermal amplification (LAMP) is a promising NAT for diagnosing leishmaniasis, including tegumentary leishmaniasis (TL). In recent years, studies employing LAMP assays with high accuracy values for TL have been published. As a globally distributed genus Leishmania parasites show significant genetic variance inside and between species and strains, even in genomic regions generally considered conserved. This variance, if located in an assay's primer target region (TR), can result in decreased accuracy. The present study aims to identify genomic variations inside an assay's TR for detecting *Leishmania (Viannia) braziliensis*. We used primers previously published for an HSP70-LAMP assay. The sequences of primers were aligned to *L. braziliensis* (taxid:5660) to determine their TRs within the genome using primer-BLAST. Next, a nucleotide-BLAST of the TR was performed to identify all *L. braziliensis* sequences available in the NCBI's GenBank database. Only mutations overlapping the TRs of the primer set were considered. Mismatches occurring in the first 5 positions of the 3' end of the analyzed primers were designated as "high risk", and the remaining variants were assigned "moderate risk". Using Megablast, 145 nucleotide sequences related to *L. braziliensis* HSP70 were found, with only one showing 100% agreement with the TR. Six relevant TRs were identified, each containing at least four polymorphisms inside primer-complimentary regions for the HSP70-LAMP assay. Most mutations were located inside the F3 and B2 primer regions, with just one polymorphic locus inside the F2 region. No "high risk" alterations were found. The next step will be to synthesize whole TRs for testing them against their respective primer set. This analysis will allow us to know the real effect of mutations on the detection limit of the primers used in HSP70-LAMP assay.

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