COMPARING FOUR DNA EXTRACTION METHODS FOR FIELD-READY MOLECULAR DETECTION OF *Leishmania* USING THE COLORIMETRIC LAMP-BASED LeishID TEST

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Abstract

Early diagnosis of leishmaniasis is crucial for disease control. The loop-mediated isothermal amplification (LAMP) method has shown great potential for point-of-care (PoC) molecular diagnostics. However, the success of the diagnosis relies on obtaining high-quality DNA, making DNA extraction a critical step. In this study, we evaluated 4 different DNA extraction methods, followed by LAMP on 84 samples from various tissues of small mammals, including liver, skin, tail tip, and ear tip. The extraction protocols used were: (i) Wizard® Genomic DNA Purification Kit, (ii) SwiftX™ DNA Kit, (iii) non-commercial boiling method, and (iv) commercial DNA extraction reagent. LAMP reactions were performed using WarmStart colorimetric master mix (NEB) that turns from pink to yellow when positive. The amplified products were also analyzed by agarose gel electrophoresis. According to the 260/280 and 260/230 absorbance ratios, the DNA obtained by method (i) showed the best quality, with values ranging from 1.1-2.9 and 0.7-3.1, respectively. Method (ii) had ratios of 0.5-1.5 and 0.2-0.8, method (iii) ranged from 1.3-1.9 and 0.4-1.3, and method (iv) had ratios of 0.9-1.6 and 0.2-0.8. Method (i) led to better DNA quality, but with a lower yield, while method (ii) had lower DNA quality and the second highest yield. Method (iii) was the most promising, offering good yield and good purity. Method (iv) had the highest yield but lower quality compared to methods (i) and (iii). Method (iii) resulted in correct amplification, suggesting that this method is promising for PoC diagnosis of leishmaniasis. Based on this, we propose a field-deployable diagnostic solution. The boiling extraction and amplification reaction will be carried out on the miniPCR device. This will be part of a portable laboratory where all equipment and supplies will fit inside a Pelican-like rigid case, enabling point-of-need molecular tests. The proof-of-concept is being performed at a veterinarian clinic.

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