Visceral leishmaniasis: improved molecular diagnosis using the mini direct on blood PCR Nucleic Acid Lateral Flow Immunoassay (dbPCR-NALFIA).

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Accurate and early diagnosis of Visceral Leishmaniasis (VL) is important to install proper treatment, because of the fatality of the condition and the high toxicity of available treatments. Current diagnostic methods include parasitology and serology (with rK39 dipstick test and direct agglutination test). These methods do have limitations (patient safety or diagnostic accuracy), and molecular testing is proposed to improve diagnosis. Current molecular tools, in particular PCR, have high accuracy for detecting VL, however their complexity and high costs make their use unsuitable for endemic areas with limited resources. Consequently, there is a need for a simple molecular diagnostic test that can be implemented in resource limited setting. We have developed a miniaturized direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA) as an innovative, easy-to-use molecular assay for the diagnosis of VL in these particular settings. Unlike other simplified molecular methods, such as LAMP, the mini-dbPCR-NALFIA does not require DNA extraction and utilizes a handheld, portable thermal cycler powered by a solar-charged power pack enabling to perform the test without any laboratory infrastructure. Reading of results is done using a rapid lateral flow strip. In the present study we have conducted a laboratory evaluation on the mini db-PCR-NALFIA to determine its diagnostic accuracy. Patient samples (N=146) with suspected VL were tested using the mini db-PCR-NALFIA and compared to conventional PCR (reference test). Sensitivity and specificity represented the accuracy. Cohen's k determined the degree or agreeableness between the mini db-PCR-NALFIA and other diagnostic tests (PCR and rk39 rapid test). Compared to qPCR, the mini db-PCR-NALFIA for VL had a sensitivity of 95.83% (95% CI, 88.30%-99.13%) and a specificity of 97.22% (95% CI, 90.32% - 99.66%). The agreement between both tests was excellent (k-value: 0.93). The Limit of Detection of the platform is around 10 parasites per microliter of blood (spiked with promastigotes). The VL-mini-db-PCR-NALFIA is now ready for large field evaluations in disease endemic countries.