

PANLEISH: A RT-qPCR PROTOCOL FOR DETECTION OF LEISHMANIA spp IN BIOLOGICAL SAMPLES

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Leishmaniasis is a chronic progressive parasitic zoonosis of high importance for public health, since it affects humans and domestic animals in its urban infection cycle. In addition, the disease can present lethal clinical manifestations, therefore deserving attention regarding prevention, diagnosis and monitoring. The diagnostic method for leishmaniasis may vary according to the clinical form and consequently the species of *Leishmania* responsible for the disease. The sensitivity and specificity of the tests may be low depending on the clinical form, with the gold standard for diagnosis being histopathological examination with direct identification of the parasite in biopsies of the lesion. In this study, we developed a unique RT-qPCR diagnostic test for the detection of *Leishmania* spp in the biological samples. For this, we identified consensus regions in the genomes of *Leishmania* spp *in silico* with a potential for designing primers for SYBR green-based RT-qPCR. DNA and RNA samples were obtained from the isolates from axenic culture of *L. infantum*, *L. braziliensis* and *L. amazonensis*. Furthermore, tissues of hamsters infected by *L. infantum* were used to validate the technique. We identified pairs of primers capable to detect 7 species of *Leishmania*: *L. donovani*; *L. major*; *L. amazonensis*; *L. mexicana*; *L. braziliensis* e *L. panamensis*, which are representatives of different clinical forms of the *Leishmaniasis*. In addition, our RT-qPCR test detected different levels of DNA/RNA from isolates of *Leishmania* spp. or the tissue samples of infected animals, but not in their blood. The test was specific for *Leishmania* spp, showing no nonspecific detection in *Trypanosoma cruzi* samples. Our data allow us to state that we have established a unique molecular diagnostic method for different *Leishmania* named here as "Pan-Leish". However, further analyses are still needed to identify the sensitivity of the molecular test developed.

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