Introduction: Leprosy is endemic to Brazil, which stands as one of the countries with the highest number of new cases every year. Socioeconomic factors, insufficiency research investments, and social stigma reinforce leprosy as a neglected disease. Those obstacles contribute to tardiness in diagnosis, which results in late treatment initialization, and, consequently, sustained disease transmission. The current work is the initial step towards the development of a portable molecular diagnostic test that would enable access to sensitive and specific diagnostic tools for vulnerable populations in regions lacking infrastructure.

Objective: To optimize and evaluate a qPCR multiplex reaction for the detection of *Mycobacterium leprae* genomic markers 16S rRNA and RLEP, as well as human 18S rRNA gene as an internal control, using the portable thermocycler Q3-Plus.

Methodology: The qPCR multiplex reaction was performed using the commercial kit NAT Hans (IBMP, Brazil) in the portable instrument Q3-Plus (Alifax, Italy). Standard dilution curves of two different samples, a synthetic DNA and DNA extracted from $10^9$ *M. leprae* cells, were used to determine the detection limit and reaction efficiency. Optical parameters such as light intensity, gain, and exposure time were optimized for each target in the Q3-Plus equipment.

Results: In the standard equipment, the reactions performed with synthetic positive control showed 94.9% of efficiency to 16S and 93.1% to RLEP, respectively, with a detection limit of 2.29 copies/uL. In Q3-Plus equipment, reactions performed with synthetic positive control were able to amplify 3.67 copies/uL, similar to published results. Reaction efficiency was estimated as 87.12% for the 16S gene target, with a LOD95% of 47.68 copies/uL. For the RLEP target, reactions in the portable instrument presented 86.89% efficiency and a LOD95% of 53.57 copies/uL. Reactions performed with DNA extracted from *M. leprae* cells showed an efficiency of 95.39% for 16S and 90% for RLEP, with an estimated LOD95% of 1013.18 genome equivalents/uL.

Conclusion: Data obtained with the portable instrument show similar efficiency and LOD95% as published results. This represents the first step towards the development of a portable molecular diagnostic test for diagnosis of leprosy in low resource environments.

Keywords: Molecular diagnosis; Point-of-Care; Leprosy