PARASITO2025
29º CONGRESSO BRASILEIRO DE PARASITOLOGIA

EVALUATION OF THE ANALYTICAL SENSITIVITY OF PRIMERS DESIGNED FOR THE NADH 1 AND NADH 5 GENES IN POLYMERASE CHAIN REACTION (PCR) FOR THE IDENTIFICATION OF *Trypanosoma cruzi*

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Abstract

Chagas disease, also known as American trypanosomiasis, is an infection caused by *Trypanosoma cruzi* that affects humans and other mammalian species. Detection of the parasite's DNA through Polymerase Chain Reaction (PCR) can be crucial for diagnosing the chronic phase of the infection due to subpatent parasitemia and in cases where conventional serology yields inconclusive results. This study aimed to determine the analytical sensitivity of primers designed for the NADH 1 and NADH 5 genes using serial dilutions of *T. cruzi* DNA extracted from cultures. The primers were designed using Primer-Blast based on DNA sequences of reference strains, clones, and isolates of *T. cruzi* deposited in the NCBI GenBank and were tested both *in silico* and *in vitro*. Subsequently, analytical sensitivity was evaluated using strains and isolates from DTUs I, II, and III, with *T. cruzi* DNA concentrations ranging from 0.001 fg to 1000 fg. After PCR amplification, the primers targeting the NADH 1 gene (5' – AAGTCCAGCAACCAATTCACTT – 3' and 3' – CGTTACTCTGTGATGGCTTGA – 5') generated a fragment of approximately 71 bp, which was detected at a concentration of 1000 fg in DTU I. These primers failed to detect DTUs II and III at any tested concentration. Conversely, the NADH 5 primers (5' – AGAGTACACAGTTTGGGTTG – 3' and 3' – CCACATACAACTAACGTTGC – 5') generated a 100-bp fragment and detected *T. cruzi* DNA at 1000 fg in DTU I and from 10 fg to 1000 fg in DTUs II and III. Thus, the NADH 1 primers were effective for detecting *T. cruzi* DTU I at high DNA concentrations, while the NADH 5 primers were efficient in identifying the parasite in DTUs I, II, and III.

Supported by: CAPES, CNPq
Keywords: *Trypanosoma cruzi*, NADH 1, NADH 5