Sensitivity of conventional polymerase chain reaction protocols used for detection and typing of *Trypanosoma cruzi*

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ABSTRACT

Chagas disease is caused by the protozoan *Trypanosoma cruzi*, being an important health problem in Brazil and Latin America. The vast majority of infected individuals are in the chronic phase, which is characterized by a low parasitemia. At this phase, molecular methods, such as polymerase chain reaction (PCR), may be an alternative to parasitological methods for detection of the parasite. In this study, we evaluated the sensitivity of different conventional PCR protocols used for detection (minicircle - PCR/330 bp and repetitive region of nuclear satellite DNA), and typing (PCR/COII and 24Sα rRNA gene) of *T. cruzi*. The DNA of the parasites belonging to DTU I (PR-150) and DTU II (PR-1256) were extracted by phenol-chloroform and chloroformisopropanol. DTU I showed higher sensitivity for most of the markers for detection and typing of *T. cruzi* evaluated, when DNA was extracted with chloroform-isopropanol. The minimum amount of parasites detected by PCR/330 was 0.00000000000002 flagellated (flg), for PCR/RFLP-COII, it was 2.36 flg and for the 24Sα rRNA gene it was 4.7 flg. The satellite DNA of DTU I had higher sensitivity with phenol-chloroform extraction, corresponding to 0.000066 flg. We concluded that the sensitivity of detection and typing of *T. cruzi* is directly related to the DTU and to the extraction method; chloroformisopropanol was the best extraction method for most of the markers used; detection methods were more sensitive than the typing methods and low intensity bands in the gel can be considered positive results.

Keywords: *Trypanosoma cruzi*. kDNA minicircle. Repetitive region-Nuclear DNA PCR/RFLP-COII. 24Sα rRNA