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Trabalhos Científicos

REPRODUCIBILITY OF K-DNA PCR ASSAY FOR DIAGNOSIS OF CHAGAS'DISEASE: PRELIMINARY STEP TO IMPLEMENT A MOLECULAR TEST IN DIAGNOSIS CLINICAL ROUTINE

Autores:
Luciana de Freitas Campos Miranda - IPEC
Veronica da Silva Santos - IPEC
Maria de Fatima Madeira - IPEC
Alejandro Marcel Hasslocher Moreno - IPEC
Pedro Emmanuel Alvarenga Americano do Brasil - IPEC
Liane de Castro - IPEC

Eixo Temático:
Diagnóstico

Introduction: For confirmation of clinical suspicion of Chagas' disease, two different technique of conventional serology tests should be applied simultaneously. In case of disagreement between the two serological tests, a confirmatory test like polymerase chain reaction (PCR) is required. The sensitivity of PCR assay is higher compared with xenodiagnosis and hemoculture. However, there is not yet available on the market a commercial kit for molecular diagnosis of Chagas disease. Several different PCR-based approaches, which aim to amplify kinetoplast DNA, have been successfully employed to diagnose T. cruzi infection in the blood of patients with Chagas' disease. However, the high variability of PCR results found in these studies raises some questions concerning its applicability for diagnosis in clinical routine. In an early phase in the process to implement Chagas' disease PCR diagnosis in clinical routine, the present study was aimed to test the reproducibility of PCR assay for the diagnosis of chronic Chagas' disease.

Methodology: This investigation was performed at Instituto de Pesquisa Clinica Evandro Chagas (IPEC) - FIOCRUZ, Rio de Janeiro, Brazil - reference center. In the period from May 2008 to November 2008, from a sequence of patients with suspicious chronic Chagas disease in clinical routine, blood samples with EDTA were tested from 60 patients. DNA was extracted from 200 mL from patients' blood samples, with a commercial kit (QIAGEN), performed as the instructions of the manufacturer. The sequences of oligonucleotides used in the PCR assay were 5'AAA TAA TGT ACG GG(T/G) GAG ATG CAT GA -3' (Primer 121) and 5'GGT TCG ATT GGG GTT GGT GTA ATA TA 3´ (Primer 122). These primers anneal in the constant regions of the minicircles of T. cruzi k-DNA for amplification of a 330 bp fragment. The amplified products were visualized by ethidium bromide staining after 1,5% agarose gel electrophoresis. To compare the reproducibility of the test, two masked professionals performed the PCR assays independently with the same samples, in the same conditions. The professionals have different expertise in molecular biology diagnostic tests. The raters classified the samples as either positive or negative. The reproducibility was estimated through agreement among the raters and kappa statistic.

Results: Initial standardization of the test showed better result with a slightly different protocol from those widely available in international literature. For example, most protocols refers to guanidine immediately added to EDTA blood samples after collection. However, guanidine seem to be unnecessary because the test is being executed in a hospital environment and thus DNA extraction was right after blood collection in EDTA tubes. DNA extraction with phenol cloroform is no longer acceptable in hospital environment due to biohazard issues and know to be much less reliable then commercial kits. From 60 blood samples tested with K-DNA-PCR assay, the results found by professional 1 showed 25 negative samples and 35 positive samples, while the results found by professional 2 showed 21 negative samples and 39 positive samples. The two professionals that performed K-DNA PCR assay obtained the same results for 52 samples; 19 negative samples and 33 positive for detection of T. cruzi. These results showed a agreement of 86%.The kappa statistic was estimated as 0.72.
Discussion: It is known that “in-house” diagnostic tests have lower reliability and thus the difference found between the raters can not be explained based only on the idea that the professional with less experience with the K-DNA PCR assay would have more difficulties in achieving better results. Even with a small sample, we believe the agreement observed between professionals 1 and 2 with the K-DNA PCR assay was satisfactory, although not perfect. The research may now go to further steps and estimate this test validity in a more clinical scenario to later fulfill Brazilian recommendations.

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