Analytical Validation of Quantitative Real-Time PCR Methods for Quantification of Trypanosoma cruzi DNA in Blood Samples from Chagas Disease Patients

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Resumen
An international study was performed by 26 experienced PCR laboratories from 14 countries to assess the performance of duplex quantitative real-time PCR (qPCR) strategies on the basis of TaqMan probes for detection and quantification of parasitic loads in peripheral blood samples from Chagas disease patients. Two methods were studied: Satellite DNA (SatDNA) qPCR and kinetoplastid DNA (kDNA) qPCR. Both methods included an internal amplification control. Reportable range, analytical sensitivity, limits of detection and quantification, and precision were estimated according to international guidelines. In addition, inclusivity and exclusivity were estimated with DNA from stocks representing the different Trypanosoma cruzi discrete typing units and Trypanosoma rangeli and Leishmania spp. Both methods were challenged against 156 blood samples provided by the participant laboratories, including samples from acute and chronic patients with varied clinical findings, infected by oral route or vectorial transmission. kDNA qPCR showed better analytical sensitivity than SatDNA qPCR with Limits of detection of 0.23 and 0.70 parasite equivalents/mL, respectively. Analyses of clinical samples revealed a high concordance in terms of sensitivity and parasitic loads determined by both SatDNA and kDNA qPCRs. This effort is a major step toward international validation of qPCR methods for the quantification of T. cruzi DNA in human blood samples, aiming to provide an accurate surrogate biomarker for diagnosis and treatment monitoring for patients with Chagas disease.

Palabras clave
KeyWords Plus: PARASITEMIA; CONSENSUS; LINEAGES; REGIONS; ASSAYS; TRIAL

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