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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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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

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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. *(J Mol Diagn 2015, 17: 605—615; http://dx.doi.org/10.1016/j.jmoldx.2015.04.010)*

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi,* affects mostly the poor populations in 21 countries of the Americas, where close to 7 to 8 million people are infected, 25 million are at risk, and 10 thousand deaths are recorded annually (World Health Organization, *www.who.int/mediacentre/factsheets/fs340/en*, last accessed November 1, 2014).[1] In recent years, this neglected tropical disease is becoming a global concern because of the increasing migration from Latin America to nonendemic countries from Europe and North America.[2]

Complex interactions between the genetic background of the parasite and the host and environmental and epidemiologic factors determine the outcome of the infection. In the acute phase of CD the symptoms are variable, and in most cases resolve spontaneously after some weeks. Appropriate treatment can eliminate the parasite during this phase, but the infection is only recognized in 1% to 2% of infected persons during the acute phase. In the chronic phase, approximately 70% of seropositive persons are asymptomatic, whereas 30% ultimately develop serious cardiac and/or digestive disorders several years or decades later, and necrotizing inflammatory injuries in the central nervous system in cases of CD reactivation under immunodepression. Each year, 2% to 3% of symptomatic persons start to present manifestations that can rapidly evolve to sudden death. However, the factors that govern the progression of chronic CD remain unknown, and no prognostic markers are available.[3]

Accurate diagnostics tools and surrogate markers of parasitologic response to treatment are priorities in CD research and development.[4] To develop an accurate laboratory tool for diagnosis and treatment follow-up, several difficulties need to be addressed, such as the low and intermittent number of circulating parasites during the chronic phase of infection and parasite genotype diversity, because six discrete typing units (DTUs), TcI to TcVI, are unevenly distributed in different endemic regions.[5] Quantitative real-time PCR (qPCR)-based assays may fill these gaps, but their application in the clinical practice requires prior analytical and clinical validation studies.[6,7] So far, a few real-time PCR strategies have been developed for *T. cruzi* DNA detection and quantification in CD patients.[8—11]

As part of the Small Grants Programme (joined initiative of Communicable Diseases Research/Pan-American Health Organization) and The Special Programme for Research and Training in Tropical Diseases/United Nations Development Program/United Nations Children's Fund/World Bank/World Health Organization, an international study was performed by 26 experienced PCR laboratories from 14 countries to assess the performance of duplex qPCR strategies on the basis of TaqMan probes for detection and quantification of the parasite loads in blood samples of CD patients.

Materials and Methods

Ethics Statement

The studies in which the samples were collected were approved by the ethical committees of the participating institutions, according to the principles expressed in the Declaration of Helsinki. Written informed consent forms were signed by the adult study subjects and from parents/guardians on behalf of all minor subjects. All samples were pre-existent at the time of this international study and were anonymized before being processed.

Spiked Blood Samples

Seronegative human blood samples were spiked with cultured epimastigotes of Sylvio X10 and CL-Brener stocks (TcId and TcVI, respectively) and were immediately mixed with one volume of guanidine hydrochloride 6 mol/L EDTA 0.2 mol/L buffer, pH 8.00 (GE).

Patients and Blood Specimens

Peripheral blood samples from 156 CD patients were distributed into eight groups according to their geographic origin, as follows. Group 1 (G1) included samples from four seropositive patients from Mexico, two patients with acute CD (G1a) and two patients with asymptomatic chronic CD (G1b). Group 2 (G2) included samples from two patients from French Guiana with acute CD acquired by oral

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