

Call for laboratories to participate in a workshop on the standardization and analytical validation of qPCR for quantification of *Trypanosoma cruzi* DNA loads in peripheral blood of Chagas disease patients.

The Small Grant Programme (CDR/PAHO/ TDR/UNICEF/UNDP/World Bank/WHO) makes a call aimed at bringing together researchers from different institutions in Chagas disease endemic and non endemic countries and providing them with an opportunity to evaluate qPCR technologies against a reference set of samples in an international workshop. The workshop will allow evaluating the performance of qPCR methods selected by the technical advisory committee. One member from each participating laboratory will be invited to a one-week workshop in Buenos Aires during the second week of December 2011. By sharing methodologies and results it is expected that this will lead to a standardization of the use of qPCR for quantification of *T. cruzi* DNA in human blood samples.

BACKGROUND AND OBJECTIVES

PCR technology has been used successfully for the diagnosis and assessment of disease progression and outcome in many infectious diseases, and has been available for analysis of clinical samples infected with *T. cruzi* from more than 15 years. However, each laboratory applied its own protocols and technologies making comparison of PCR findings not reliable among these laboratories. In this context, three years ago, TDR UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases), and PAHO/WHO promoted the standardization and validation of PCR for diagnosis and disease management of Chagas disease across laboratories and countries. Accordingly, a project proposal was developed to validate and harmonize PCR methods for detection of *T. cruzi* DNA in human blood samples. A study protocol was developed: Blinded blood samples with known number of parasites, purified DNA from strains belonging to different *T. cruzi* genetic lineages and relevant controls were prepared by the coordinating centre and sent to 29 centres all over the world that had responded to the call. Each laboratory performed the PCR analysis according to its own protocols and using its own financial resources. Results were submitted to the coordinating laboratory, and those laboratories that fulfilled the minimum requirements of concordance with the content of the samples provided were invited to participate in a four-day "hands-on" workshop that was held at INGEBI, Buenos Aires, Argentina followed by a two-day Symposium (17 to 22 November 2008). Representatives from Argentina, Belgium, Bolivia, Brazil, Chile, Colombia, France, French Guyana, Paraguay, Peru, Spain, United Kingdom and Uruguay participated in this workshop. The workshop allowed defining the best PCR practices as well as their applicability in a clinical setting and interpretation of results. The conclusions of the study as well as the methods selected to elaborate a Standard operative procedure for PCR applied to *T.cruzi* detection were published in Schijman, AG et al, PLoS NTD, 2011. Among the methods that performed best, there were a few based on Real-Time PCR targeting satellite and

kinetoplastid DNA sequences, which had the potential for being used for quantitative purposes (qPCR). These qPCR assays could possibly play a key role in the context of evaluating new drug candidates in clinical studies.

Accordingly, PAHO, WHO and TDR decided to support a follow-on phase of this initiative, which is now focused on the analytical validation of selected qPCR technologies based on satellite and kinetoplastid DNA sequences which have been improved and standardized by members of the technical adviser committee through the incorporation of internal standards in a multiplex Format using TaqMan Probes, as follows,

- Method I - Real Time Multiplex PCR using TaqMan probe directed to satellite sequence (primers Cruzi 1, Cruzi 2, probe cruzi 3) (Piron et al, 2007) plus exogenous or endogenous internal amplification control .
- Method II - Real Time Multiplex PCR using TaqMan targeted to KDNA sequences (primers 32f, 148r, conserved region, LNA TaqMan probe 71p (See Method LbF2 in Schijman et al, PLoS 2011) plus exogenous or endogenous internal amplification control.

qPCR workshop activities:

The aim of the practical workshop is to challenge DNA extraction and qPCR methods with a panel of characterised samples, done in the same laboratory by different operators using the same DNA extraction protocols, qPCR reagents and cycling conditions. A representative from each selected participant laboratory will be able to test the proposed protocols in a common venue, allowing estimation of the performance of each assay, leading to the preparation of common standard operating procedures (SOPs).

Performance characteristics will be assessed from a) serial dilutions of DNA samples from reference *T.cruzi* cultured stocks belonging to different Discrete Typing units mixed with human DNA, and b) from blood samples spiked with serial dilutions of *T.cruzi* cells, following recommendations from recognised international guidelines (CLSI, Burd, E; Clin.Microbiol.Rev, 2010),.

PRELIMINARY SCHEDULE

<p>Day 1</p>	<p>Opening Session</p> <p>9.00 - 9.30 - Reception to the Workshop Mirta Flawiá, Zaida Yadón, Piero Olliaro , Alejandro Schijman</p> <p>9.30-10:30 - PRESENTATION OF PROPOSED qPCR-METHODS Description of principles of each DNA extraction method and qPCR procedure to be performed by the participants during the workshop. Each participant will test aliquots of the same DNA and spiked blood samples. Among them, there are artificial blood samples infected with T. cruzi from different lineages covering a range of parasitic loads. Internal Amplification Standards allowing follow-up of the whole procedure, as well as controls to assess analytical sensitivity and specificity of the amplification will be distributed.</p> <p>10.30-10.45 - Coffee break</p> <p>10.45-13.00 - Manipulation of Samples DNA Extraction procedures Each participant will process each sample using commercial kits based on the use of columns. DNA extracts will be stored for qPCR.</p> <ul style="list-style-type: none"> • 13:00-14:00: Lunch • 14:00-20.00 Master Mix preparation, amplification and analysis of Results. Staff of the Laboratory will prepare the standard curve to evaluate qPCR efficiency.
<p>Day 2</p>	<p>First Manipulation to evaluate reproducibility</p>
<p>Day 3</p>	<p>Second Manipulation to evaluate reproducibility</p>
<p>Day 4</p>	<p>Third Manipulation to evaluate reproducibility</p>
<p>Day 5</p>	<p>Analysis and discussion of results Elaboration of Technical Report with discussion and conclusion of the Workshop. Planning of clinical validation and proficiency testing.</p>

Public and private laboratories currently using qPCR techniques for Chagas disease research are invited to participate. Only laboratories meeting the following requirements shall apply:

1. To agree that the technical procedures related to the qPCR methodologies being evaluated during the workshop will be made publicly available (Please provide a short statement).
2. To demonstrate experience in processing clinical samples for qPCR based detection of *T. cruzi* DNA, providing relevant publications on the development, validation and/or use of qPCR for Chagas disease research (Please provide PDF copy of publications or others such as abstracts) Priority will be given to those laboratories involved in follow-up of Chagas disease patients under treatment with parasitic drugs.-
3. To commit to future laboratory work to detect *T. cruzi* in human clinical samples as needs will emerge following the process of qPCR validation.

Interested laboratories should provide a brief letter of intent addressing the above mentioned requirements to:

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Dateline: October 16, 2011. No application would be received after this data.

Funding: travel, lodging and laboratory expenses would be available to cover some selected participants from selected South and Central American countries
