Evaluation of RealStar® Chagas PCR Kit 1.0 for qualitative detection of *Trypanosoma cruzi* DNA in whole blood samples of Chagas disease patients

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Introduction

Chagas disease (CD) caused by an infection with *Trypanosoma cruzi* is most likely "the most neglected of the neglected diseases" (WHO, 2012) [1; 2]. It has been looked at as an endemic disease in tropical and subtropical areas of Southern and Central America, Mexico and Southern United States, but lately it has been recognized as an emerging global distress in non-endemic areas [3; 4].The infection traverses an acute phase, evolving to an either asymptomatic or symptomatic chronic phase, with different degrees of progression and severity [5].

Molecular Diagnostic of Chagas Disease – Background:

Molecular diagnosis of *T. cruzi* is important for early detection of congenital transmission in newborn when presence of maternal anti-*T. cruzi* antibodies persists long time and microscopic observation lacks sensitivity. In addition, there is an urgent need for PCR diagnosis of oral infections, early detection of infection in organ recipients from CD donors, monitoring of *T. cruzi* reactivation in chronically infected immunocompromised patients and evaluation of treatment response, since detection of serological negative conversion in treated patients with a favorable outcome may take many years to occur [2].

Aim of Study:

The aim of this study was the validation of the RealStar® Chagas PCR Kit 1.0 in comparison with an established *in-house* workflow for the detection of *Trypanosoma cruzi* specific DNA at the Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, by analyzing 96 individual whole blood samples from patients under suspicion of *T. cruzi* infection.

Methods

Nucleic acids were extracted from 200µl of each whole blood sample, using the manual HighPure PCR Template Preparation Kit (Roche) following the instructions provided in "Instructions for Use" and normalized by multicenter study. The Internal Control provided with the RealStar® Chagas PCR Kit 1.0 was added to the extraction as described in the respective "Instructions for Use". The PCR was performed on Rotor-Gene® Instrument (QIAGEN) accordingly.

In parallel, the same eluates were used for conventional PCR analysis with the *in-house* kDNA PCR assay established at the Centro Nacional de Microbiología, Instituto de Salud Carlos III, based on the publications from Norman *et al.* (2011) and Ramírez *et al.* (2015) [6; 7]. Samples were tested in duplicate. A sample was positive if at least one replicate had a positive result.

For diagnostic evaluation a total of 96 individual whole blood samples were analyzed retrospectively. Samples were collected in consideration of clinical symptoms of Chagas Disease as well as their serological status.

Results

68 out of 96 patients included in this study had Chagas disease confirmed by clinical symptoms. 28 out of the 96 patients were tested serological negative for *T. cruzi*.

In 64 out of 68 patients with confirmed Chagas disease, *Trypanosoma cruzi* DNA was detected with the conventional *in-house* kDNA PCR reference assay, whereas 60 out of these 68 patients were tested positive for *T. cruzi* DNA using the RealStar® Chagas PCR Kit 1.0.

The 28 serological negative samples were tested negative for *T. cruzi* DNA with both assays.

Table 1: Comparison of qualitative PCR results by pre-characterization of samples

No. of Patients tested clinically and serologically for Chagas Disease [CD]: 96		Conventional *in-house* kDNA PCR assay	RealStar® Chagas PCR Kit 1.0
No. of Patients with acute / reactive CD	13	13	13
No. of Patients with congenital CD	14	14	14
No. of Patients with chronic CD	41	37	33
No. of negative Patients	28	28	28

Based on this study, the diagnostic sensitivity and specificity of the RealStar® Chagas PCR Kit 1.0 for the detection of *T. cruzi* DNA in relation to the conventional *in-house* kDNA PCR assay based on Norman *et al.* (2011) and Ramírez *et al.* (2015) is 94% and 100%, respectively.

Discussion

During the chronic phase of Chagas disease the parasite concentration in the blood is very low. Diagnosis of chronic Chagas disease is therefore made after consideration of the patient's clinical findings, as well as by the likelihood of being infected, such as having lived in a country where Chagas disease is common. Diagnosis is generally made by testing for parasite specific antibodies in the patient, since during the chronical phase of infection molecular assays often struggle with sensitivity.

Testing multiple replicates of such samples by PCR and/or serial sampling can improve the positive detection by molecular assays of such patients: Collection of several serial blood samples and PCR triplicates for each sample are in many laboratories the strategy to address this problem. A patient can be considered *T. cruzi* positive if at least one PCR replicate was detectable. Disadvantages of this approach are time and labor intensity, as well as the danger of contamination when using conventional PCR.

In this study the discrepant results are based on samples from patients with chronic Chagas disease. Also here, pre-characterization of the samples was based on clinical and serological findings. PCR is used in such cases for confirmation of the disease and multiple testing remains the most reliable way for this.

Conclusion

The study shows the suitability of the commercial RealStar® Chagas PCR Kit 1.0 for qualitative detection of *Trypanosoma cruzi* DNA in whole blood samples especially for reactive / acute and congenital CD patients. Diagnostics of the chronic status of Chagas Disease by molecular assays still stays a challenge. Based on clinical and serological findings in the patient, confirmation of the chronic status of the disease by PCR may use several samples for testing and/or running the PCR in multiple repeats.

References

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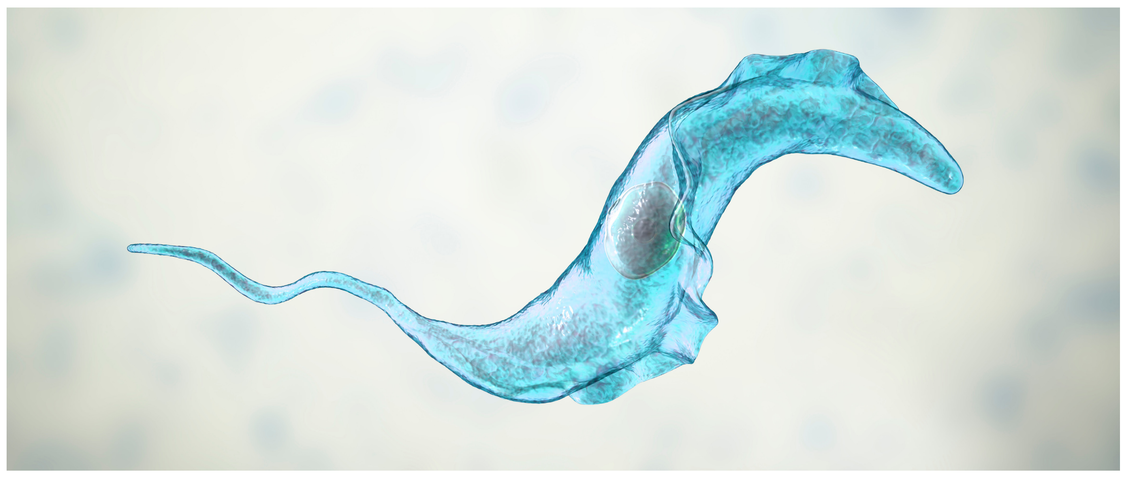

Figure 1: *Trypanosoma cruzi*