New real-time PCR assay for the detection of *Trypanosoma cruzi*

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Introduction

Chagas disease is a vector-borne parasitic and potentially life-threatening illness caused by the protozoa *Trypanosoma cruzi.* The disease is endemic in South and Central America with 6 to 7 million people estimated to be infected worldwide. *Trypanosoma cruzi* is mainly transmitted to humans by contact with feces of infected triatomine bugs. Infections can also occur through blood transfusion, organ transplants, congenitally or orally. Chagas disease presents itself in two phases. The initial and acute phase is usually characterized by mild or no symptoms and parasitaemia in circulating blood. The chronic phase may last throughout life or evolve into clinical manifestation. A standardized sensitive and specific diagnostic assay is of great necessity for the reliable diagnosis of Chagas disease because it can lead to a fatal outcome for the patient. Here we report the analytical and diagnostic evaluation of a real-time PCR assay developed for the detection of *Trypanosoma cruzi (T. cruzi)* specific DNA, the RealStar® Chagas PCR Kit 1.0.

Methods/Materials

In addition to the specific detection of *T. cruzi* specific DNA, an Internal Control (IC) system was implemented. The IC allows monitoring of the efficiency of the nucleic acid extraction process and possible inhibitory effects during PCR. *T. cruzi* specific detection is in the FAM-Channel and the IC signal in the Joe/VIC-Channel (figure 2).

The analytical sensitivity (Limit of Detection: LoD) is defined as the concentration (copies/µl) of *T. cruzi* specific DNA molecules that can be detected with a positivity rate of ≥ 95%. The analytic Limit of Detection (LoD) of the specific *T. cruzi* detection system was determined by testing half-logarithmic dilutions of quantified DNA.

To analyze potential cross-reactivity of the RealStar® Chagas PCR Kit 1.0, DNA from different closely related protozoa were tested (table 1).

For the diagnostic evaluation 20 blinded whole blood samples, previously tested and described (1, 2) were tested with the RealStar® Chagas PCR Kit 1.0. DNA from the whole blood samples, pre-mixed 1:1 in pre-lysis/storage buffer, was extracted manually using the QIAmp DNA Blood Mini Kit (Qiagen). During nucleic acid extraction the IC-Template was added.

Results/Conclusion

Analytical sensitivity of the RealStar® Chagas PCR Kit 1.0 determined by Probit analysis is LoD 0.34 copies/µl [CI 0.17- 1.29 copies/µl] (figure 1).

Analytical specificity testing of the RealStar® Chagas PCR Kit 1.0 with high concentrations of DNA of different pathogens (table 1), showed no cross-reactivity except with *Trypanosoma rangeli* (table 1).

Clinical evaluation: The results for all clinical samples tested using the RealStar® Chagas PCR Kit 1.0 were concordant with the results of the reference São Paulo PCR (table 2 and figure 3). The diagnostic sensitivity and specificity were 100% (figure 3, true positives: 15/15 and true negatives: 5/5). The detailed results are summarized in table 2.

The RealStar® Chagas PCR Kit 1.0 is at least as sensitive as the reference PCR.

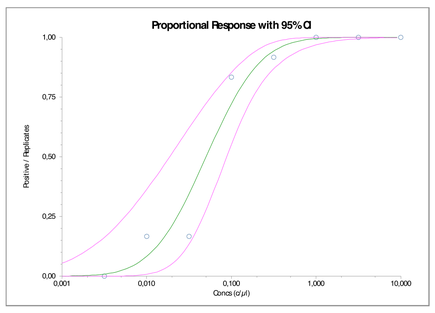

Figure 1: Graphical depiction of results of the Probit Analysis

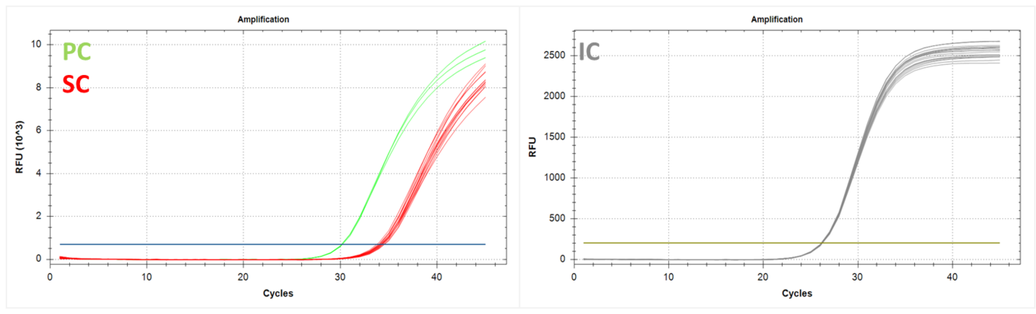

Figure 2: Set-up of assay controls

Amplifications plots of *Trypanosoma cruzi* specific controls **(A)** Positive Control (PC); Sensitivity Control (SC): ~3x LoD **(B)** Internal Control (IC) signals.

Table 1: Specificity testing of the RealStar® Chagas PCR Kit 1.0, using high concentrations DNA of the respective pathogens

Name and Concentration	FAM Channel (Ct values)	Internal Control (IC)
Trypanosma cruzi Positive Control	positive	valid
Trypanosoma brucei	– –	valid
Trypanosoma rangeli	positive	valid
Toxoplasma gondii	– –	valid
Leishmania major	– –	valid
Leishmania donovani	– –	valid
Leishmania infantum	– –	valid
Leishmania brasiliensis	– –	valid
Plasmodium falciparum	– –	valid
Plasmodium vivax	– –	valid
Plasmodium malariae	– –	valid
Plasmodium ovale	– –	valid
Plasmodium knowlesi	– –	valid

		RealStar® Chagas PCR Kit 1.0	
		POSITIVE	NEGATIVE
Reference PCR	POSITIVE	15	0
	NEGATIVE	0	5

Figure 3: Results of retrospective testing of blinded whole blood samples

All samples tested showed concordant results, using the RealStar® Chagas PCR Kit 1.0 and the reference São Paulo PCR.

Table 2: Comparison of results from testing 20 whole blood samples with RealStar® Chagas PCR Kit 1.0 and the reference PCR (Universidade de São Paulo)

	RealStar® Chagas PCR Kit 1.0			São Paulo PCR	
Sample No.	Mean Ct Values	Internal Control	Results	PCR parasites/20mL	Results
SPChagas 1	36.16	valid	positive	16.82	positive
SPChagas 2	32.78	valid	positive	33.64	positive
SPChagas 3	33.89	valid	positive	1.05	positive
SPChagas 4	31.84	valid	positive	16.82	positive
SPChagas 5	31.34	valid	positive	11.89	positive
SPChagas 6	32.01	valid	positive	5	positive
SPChagas 7	29.91	valid	positive	7.07	positive
SPChagas 8	33.21	valid	positive	11.89	positive
SPChagas 9	31.96	valid	positive	11.89	positive
SPChagas 10	32.30	valid	positive	14.14	positive
SPChagas 11	33.30	valid	positive	10	positive
SPChagas 12	– –	valid	negative	0	negative
SPChagas 13	– –	valid	negative	0	negative
SPChagas 14	– –	valid	negative	0	negative
SPChagas 15	– –	valid	negative	0	negative
SPChagas 16	– –	valid	negative	0	negative
SPChagas 17	32.04	valid	positive	1.77	positive
SPChagas 18	33.59	valid	positive	1.05	positive
SPChagas 19	33.87	valid	positive	2.97	positive
SPChagas 20	34.45	valid	positive	1.05	positive

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