Introduction

Chagas disease is a vectorborne 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 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.

Acknowledgements

A special thanks to Prof. Dr. Ester Cedeira Sabino and Léa Campos de Oliveira from Instituto de Medicina Tropical de São Paulo (Universidade de São Paulo) for providing the samples and sharing the results.

References


Margaret Nasimiyu Weber¹, Léa Campos de Oliveira², Leonie-Sophie Hecht¹ and Ester Cedeira Sabino²

altona Diagnostics GmbH, Hamburg, Germany; Universidade de São Paulo, Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil

Introduction

Chagas disease is a vector-borne parasitic and potentially life-threatening illness caused by the protozoan 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 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.

Acknowledgements

A special thanks to Prof. Dr. Ester Cedeira Sabino and Léa Campos de Oliveira from Instituto de Medicina Tropical de São Paulo (Universidade de São Paulo) for providing the samples and sharing the results.

References