altona DIAGNOSTICS

Instructions for Use

RealStar®
Chagas PCR Kit 1.0

04/2022 EN

RealStar®

RealStar®

Chagas PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

CE

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1. Intended Use

The RealStar® Chagas PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of *Trypanosoma cruzi* specific DNA.

2. Kit Components

Table 1: Kit Components

Lid color	Component	Number of vials	Volume [µl/vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Chagas PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage at +2 °C to +8 °C should not exceed a period of 2 hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

Chagas disease is a vector-borne parasitic infection caused by *Trypanosoma cruzi (T. cruzi)*. The protozoan parasite belongs to the order Trypanomastida, which compromise unicellular, flagellated obligate parasites. Chagas is endemic in South and Central America, but 6 to 7 million people are estimated to be infected worldwide **[1]**. The protozoan is transmitted to humans by contact with faeces of infected triatomine bugs, which feed during the night on the sleeping host. Infections can also occur orally **[2]**, congenitally **[3]** or through transfusions of contaminated blood or organ transplants **[4,5]**. Chagas disease presents itself in two phases: acute and chronic. The acute phase lasts for about two months and is usually an asymptomatic self-limited febrile illness characterized by high parasitaemia in the circulating blood. If signs and symptoms occur, they are usually mild and may include: swelling at the infection site, fever, fatigue, rash, eyelid swelling (Romanas's sign), headache, nausea, diarrhea or vomiting, swollen glands, enlargement of liver or spleen **[6]**. If the acute phase is not diagnosed and left untreated, the infection persists and might advance to the chronic phase. The chronic phase may last throughout life without causing symptoms or evolve into a clinical manifestation in 10%-30% of patients. Signs and symptoms of the chronic phase of Chagas disease may occur 10 to 20 years after initial infection, or they may never occur. In severe cases, however, Chagas disease signs and symptoms may include: Irregular heartbeat, congestive heart failure, sudden cardiac arrest, difficulty swallowing due to enlarged esophagus, abdominal pain or constipation due to enlarged colon **[6]**. There is no defined gold standard for the diagnosis of Chagas disease. In the acute phase of the disease, the parasites load in the circulating blood is high and Chagas disease can be diagnosed by microscopy of Giemsa-stained blood smears **[7]**. At the Center of Disease Control and Prevention (CDC) molecular detection of *T. cruzi* DNA is currently performed using a combination of three different real-time PCR assays. Molecular diagnosis of Chagas disease is performed in cases of a suspected transmission via blood transfusion or transplants and for congenital Chagas. Molecular detection can also be useful for early detection of *T. cruzi* in blood donations and transplant-transmitted recipients of organs from donors with chronic asymptomatic Chagas disease **[8]**.

[1] World Health Organization (WHO) Chagas disease (American trypanosomiasis) Geneva: WHO; 2010.

[2] Nobrega AA, García MH, Tatto E, Obara MT, Costa E, Sobel J, et al. Oral transmission of Chagas disease by consumption of acai palm fruit, Brazil. Emerg Infect Dis. 2009;15:653–5.

[3] Gürtler RE, Segura EL, Cohen JE Congenital transmission of Trypanosoma cruzi infection in Argentina. Emerg Infect Dis. 2003;9:29–32.

[4] Tropical Disease Research, World Health Organization. Insect vectors and human health. Report of the scientific working group meeting. Geneva. Organization. 2003;23–5.

[5] Grant IH, Gold J, Wittner M, Tanowitz H, Nathan C, Mayer K, et al. Transfusion-associated acute Chagas disease acquired in the United States. Ann Intern Med. 1989;111:849–51.

[6] World Health Organization (WHO), Chagas disease (American trypanosomiasis) (01.02.2018): Fact-Sheets –Chagas disease (American trypanosomiasis). http://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis). viewed 02 October 2018.

[7] Bonomo, R. & Salata, R. (2000). American Trypanosomiasis (Chagas's Disease: Trypanosoma cruzi). In R. Behrman, R. Kliegman, & H. Jenson, (Eds.), *Nelson Textbook of Pediatrics.* 16th Edition (pp. 1046-1048). Philadelphia: W. B. Saunders.

[8] Alonso-Padilla J, Gallego M, Schijman AG, Gascon J. (2017). Molecular diagnostics for Chagas disease: up to date and novel methodologies. Expert Rev Mol Diagn 17(7):699-710.

6. Product Description

The RealStar® Chagas PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of *Trypanosoma cruzi* specific DNA.

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

Probes specific for *T. cruzi* DNA are labeled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of *T. cruzi* specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labeled probes

The RealStar® Chagas PCR Kit 1.0 consists of:

- Master A
- Master B
- Internal Control
- Positive Control
- Water (PCR grade)

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of *T. cruzi* specific DNA and the Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® Chagas PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.

- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Chagas PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMAG® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® Chagas PCR Kit 1.0 has to be validated by the user.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 1 min at approximately 17,000 x g (~ 13,000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION	
	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.***

CAUTION	
	***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Chagas PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

► If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

- ► If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- ► No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.
- ► If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that at least one Positive and one Negative Control is used per run.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® Chagas PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

- ► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	None

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector name	Reporter	Quencher
T. cruzi specific DNA	*T. cruzi*	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

Depending on the real-time PCR instrument the fluorescence channel to detect the JOE™ fluorophore is not called "JOE™", but e.g. "VIC™" or "Yellow". Please refer to Table 2 for information on the fluorescence channel to be selected for the detection of the Internal Control included in the RealStar® Chagas PCR Kit 1.0.

Table 2: Detector channel to be selected for the detection of the Internal Control in dependence of the real-time PCR instrument used

Real-time PCR instrument	Detector channel for the Internal Control
Applied Biosystems™ 7500 Real-Time PCR Systems (Applied Biosystems)	JOE™
Applied Biosystems™ 7500 Fast Real-Time PCR Systems (Applied Biosystems)	JOE™
CFX96™ Real-Time PCR Detection System/ CFX96™ Dx System (Bio-Rad)	VIC™
CFX96™ Deep Well Real-Time PCR Detection System/ CFX96™ Deep Well Dx System (Bio-Rad)	VIC™
LightCycler® 480 Instrument II (Roche)	Ex: 498/533 nm; Em: 580 nm
Mx 3005P™ QPCR System (Stratagene)	JOE™
Rotor-Gene® 6000 (Corbett Research)	Yellow
Rotor-Gene® Q5/6 plex Platform (QIAGEN)	Yellow
VERSANT® kPCR Molecular System AD (Siemens Healthcare)	JOE™

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	58	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For the analysis of real-time PCR results a threshold has to be set for each fluorescence detection channel individually. To set the threshold it should be dragged into the exponential area of the amplification curve as shown in Figure 1. For more details on setting a threshold, please refer to the user manual of the respective real-time PCR instrument. The threshold cycle value (also called crossing point or quantification cycle) is the point where the threshold intersects the amplification curve.

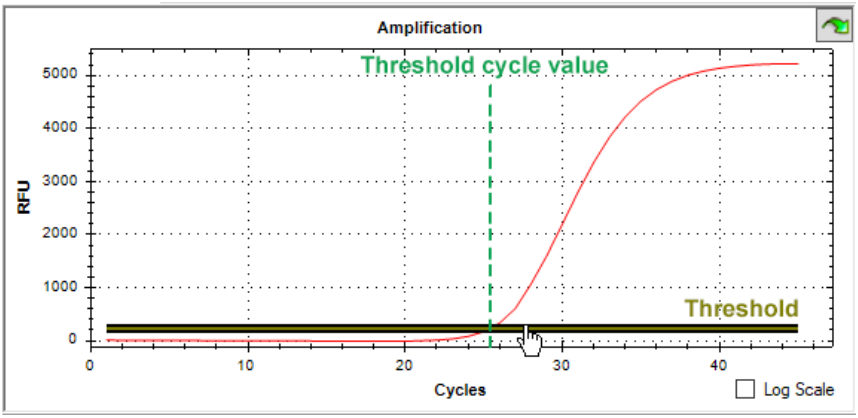

Figure 1: Setting the threshold for a fluorescence detection channel

Positive signals are represented by a number of cycles. Every numeric value above 0 and below 45 indicates a positive signal. Cycler dependent the number of cycles will be given in C_t (threshold cycle), C_p (crossing point) or C_q (quantification cycle). Positive results typically show a sigmoidal (i.e. "S"-shaped) curve. For the specific depiction of a positive signal please refer to the user manual of the respective real-time PCR instrument.

Negative signals are represented e.g. as "N/A" (means not available), abscence of a numeric value, undeterminant or abscence of numeric digital numbers in general. For the specific depiction of a negative signal please refer to the user manual of the respective real-time PCR instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Chagas PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection channel	
	FAM™	JOE™
Positive Control	C_t < 37	C_t < 40 or no C_t*
Negative Control	No C_t	C_t < 40

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection channel		Result interpretation
FAM™	JOE™	
C_t < 45[1]	Any or no C_t*	*T. cruzi* specific DNA detected.
No C_t	C_t < 40	No *T. cruzi* specific DNA detected. Sample does not contain detectable amounts of *T. cruzi* specific DNA.
No C_t	C_t > 40 or no C_t	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high *T. cruzi* DNA load in the sample can lead to a reduced or absent Internal Control signal.

[1] *Trypanosoma rangeli* is a non-human pathogenic *Trypanosoma* species, having the same prevalence and transmission route as *Trypanosoma cruzi*. Due to the assay design *Trypanosoma rangeli* positive samples generate a positive signal in the FAM™ channel.

11. Performance Evaluation

Performance evaluation of the RealStar® Chagas PCR Kit 1.0 was done using a *Trypanosoma cruzi* specific PCR product.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Chagas PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of *T. cruzi* specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of a dilution series of *T. cruzi* specific PCR product containing the target region (kDNA) used by the RealStar® Chagas PCR Kit 1.0.

Table 3: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *T. cruzi* specific DNA

Input conc. [copies/µl]	Number of replicates	Number of positives	Hit rate [%]
31.600	24	24	100.0
10.000	24	24	100.0
5.000	24	24	100.0
3.160	48	46	95.8
2.500	24	23	95.8
1.500	24	9	37.5
1.000	48	2	4.2
0.316	24	0	0.0
0.100	24	0	0.0
0.032	24	0	0.0
0.010	24	0	0.0
0.003	24	0	0.0

The analytical sensitivity of the RealStar® Chagas PCR Kit 1.0 was determined by probit analysis:

- For the detection of *T. cruzi* specific DNA, the analytical sensitivity is 2.8 copies/µl [95% confidence interval (CI): 2.5 - 3.4 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® Chagas PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant *T. cruzi* genotypes will be detected.

The analytical specificity of the RealStar® Chagas PCR Kit 1.0 was evaluated by testing a panel of genomic RNA/DNA extracted from pathogens related to *T. cruzi* and other pathogens causing similar symptoms as *T. cruzi*.

The RealStar® Chagas PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Chikungunya virus
- Dengue virus
- Human immunodeficiency virus 1
- Influenza A virus
- Influenza B virus
- West Nile virus
- *Babesia microti*
- *Leishmania donovani*
- *Leishmania infantum*
- *Leishmania major*
- *Leishmania tropica*
- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium ovale*
- *Plasmodium malariae*
- *Plasmodium knowlesi*
- *Toxoplasma gondii*
- *Trypanosoma brucei*

11.3 Precision

Precision of the RealStar® Chagas PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) values. At least 6 replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 4: Precision data for the detection of *T. cruzi* specific DNA

T. cruzi	Average threshold cycle (C_t)	Standard deviation	Coefficient of variation [%]
Intra-Assay Variability	26.82	0.11	0.40
Inter-Assay Variability	27.14	0.28	1.04
Inter-Lot Variability	26.85	0.09	0.34
Total Variability	27.03	0.28	1.04

Table 5: Precision data for the detection of the Internal Control

Internal Control	Average threshold cycle (C_t)	Standard deviation	Coefficient of variation [%]
Intra-Assay Variability	24.91	0.08	0.31
Inter-Assay Variability	24.99	0.07	0.28
Inter-Lot Variability	24.92	0.07	0.28
Total Variability	24.96	0.08	0.32

11.4 Diagnostic Evaluation

The RealStar® Chagas PCR Kit 1.0 was evaluated in a comparative study with the in-house conventional kDNA PCR assay [based on Norman *et al.* (2011) and Ramírez *et al.* (2015)]. Retrospectively, 55 individual whole blood samples were tested.

The RealStar® Chagas PCR Kit 1.0 and the in-house conventional kDNA PCR [based on Norman *et al.* (2011) and Ramírez *et al.* (2015)] were used in combination with the High Pure PCR Template Preparation Kit (Roche).

For the qualitative analysis all samples with an invalid result for one or both assays were excluded.

Results for the remaining 55 samples are shown in Table 6.

Table 6: Results of the evaluation of the diagnostic sensitivity and specificity of the RealStar® Chagas PCR Kit 1.0

		In-house conventional kDNA PCR assay [based on Norman *et al.* (2011) and Ramírez *et al.* (2015)]	
		POSITIVE	NEGATIVE
RealStar® Chagas PCR Kit 1.0	POSITIVE	27	0
	NEGATIVE	0	28

The diagnostic sensitivity and specificity of the RealStar® Chagas PCR Kit 1.0 compared to the in-house conventional kDNA PCR assay [based on Norman *et al.* (2011) and Ramírez *et al.* (2015)] for the detection of *Trypanosoma cruzi* DNA in patients with acute, reactive, or congenital Chagas disease were 100 % (confidence interval: 87.23 % to 100 %) and 100 % (confidence interval: 87.66 % to 100 %), respectively.

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the *T. cruzi* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The very low parasitemia associated with chronic *Trypanosoma cruzi* infection may lead to false negative results.
- As with any diagnostic test, results of the RealStar® Chagas PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® Chagas PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); NucliSENS®, easyMAG® (bioMérieux); CFX96™ (Bio-Rad); Maxwell® (Promega); MinElute®, QIAamp®, QIAsymphony®, Rotor-Gene® (QIAGEN); FAM™, JOE™ VIC™(Thermo Fisher Scientific); LightCycler® (Roche); VERSANT® (Siemens Healthcare); Mx 3005P™ (Stratagene).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® Chagas PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Catalogue number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade item number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution: Highlights operating instructions or procedures which, of not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics Technical Support for assistance.

Symbol	Explanation
i	Note: Information is given to the user that is useful but not essential to the task at hand.
	Version

Notes:

always a drop ahead.

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