T. cruzi DNA test

Method for *Trypanosoma cruzi* parasite DNA detection by Real-time Polymerase Chain Reaction

SUMMARY

Chagas disease (American trypanosomiasis) is an infection caused by the *Trypanosoma cruzi* (*T. cruzi*) parasite, which is an important cause of morbidity and mortality in Latin America, generating between 13,000 and 45,000 deaths of infected patients per year. Although infection is primarily transmitted by blood-sucking vectors, another important route of infection is congenital transmission from mother to fetus.

Laboratory diagnosis depends on the stage of the disease. During the acute phase, because high parasitemia generally occurs, the diagnosis is performed by direct parasitological methods. One of the most widely used direct methods is the microhematocrit technique. However, this methodology is highly operator-dependent and has low sensitivity (around 50%). Molecular methods are being incorporated into the routine for early detection of Chagas disease (before 10 months). One of the most accepted molecular methods for routine diagnosis in general is the polymerase chain reaction, and real-time PCR, which has high sensitivity. During the chronic phase, serological methods such as: immunoenzymatic assay, hemagglutination or immunofluorescence are used.

Early diagnosis allows for more successful therapeutic decisions, since it has been shown that the earlier trypanosomicide treatment is given to an infected child, the sooner seroconversion is achieved (the current consensus criteria for cure).

This diagnostic kit is intended for the identification of *T. cruzi* DNA in newborn infants of infected mothers (congenital transmission), although it is not exclusive.

It could be extended to other acute patients (vector-infected, transplanted, transfused, accidental laboratory transmission). This group of individuals is characterized by a much higher level of parasitemia than in individuals with chronic infections.

PRINCIPLE

The method is based on an in vitro nucleic acid amplification test, for detection of *T. cruzi* DNA sequences in human blood using specific TaqMan®-type probes, using Polymerase Chain Reaction technology in Real Time (qPCR).

The complete procedure consists of purifying DNA from a whole blood sample, followed by amplification of specific regions of the pathogen's genome. The amplified product is detected through fluorochromes, which are linked to probes that specifically bind to the sequence that is amplified. The fluorescence emitted during the amplification, makes possible the detection of the amplified product.

The function of each component of the reaction is as follows:

1) The *T. cruzi* reaction mix (***T cruzi* qPCR Master Mix UDG 2X** reagent) contains the Taq DNA polymerase enzyme, reaction buffer, deoxynucleotides (dNTPs), primers and probes specific for *T. cruzi* and an internal control (IAC), salts, stabilizers and preservatives, in addition to the components of the contaminant removal system by amplicon entrainment (previous amplifications). *T. cruzi* primers and probe (included in the *T cruzi* qPCR Master Mix UDG 2X) allow detection of parasite-specific DNA through the FAM channel. The probe is of the TaqMan® type (5'FAM, 3'BHQ1), which is cleaved during amplification by separating the fluorophore from the quencher. The increase in fluorescence resulting from accumulation of the tempered DNA is detected by the real-time PCR instrument. The primer sequences are highly homologous with a wide variety of clinically relevant reference sequences, based on bioinformatic analysis. These sequences belong to the highly repetitive satellite region of the *T. cruzi* genome, which is highly preserved throughout all *T. cruzi* genotypes or discrete typing units.

2) *T. cruzi* Positive Control (**PC *T cruzi*** reagent) is used to run strong and weak amplification controls in each assay, starting with serial dilutions of the PC *T cruzi* reagent. Positive fluorescence signals at appropriate values ensure the integrity and reliability of the test. The positive control at both levels serves as a control of adequate amplification and integrity of reagents. Furthermore, the weak positive control, being in the zone of the test detection limit, ensures the adequate sensitivity of the system.

3) When DNA extraction is performed from the clinical sample (EDTA whole blood/preservative), an Internal Amplification Control (**IAC**) is added, which is an exogenous source of DNA that is co-purified with the sample. It is a control of the DNA extraction process and indicates the absence of inhibitors in the amplification reaction. For this, primers and an IAC probe are used (included in the *T cruzi* qPCR Master Mix UDG 2X), in which the primers are in a limit concentration such that they allow a multiple reaction with the quench primers. The probe of this mixture is of the TaqMan® type (5'YY, 3'BHQ1), detected through the VIC channel.

4) It is also necessary to include a Negative Reaction Control (**NC**) to confirm the absence of contamination of the reagents. For this, nuclease-free water (**Nuclease-free H_2O** reagent) is used as sample. In case of a positive result for this control, the test must be repeated, previously looking for possible sources of contamination and discarding them.

PROVIDED REAGENTS

***T cruzi* qPCR Master Mix UDG 2X:** reaction mix for amplification/detection of *T. cruzi* DNA and IAC by real-time PCR in duplex. Contains: 40 mM Tris buffer pH 8.1, 100 mM KCl, 6 mM $MgCl_2$, 2 U/reaction Taq DNA polymerase, 0.4 mM dNTP's (including dUTP), 0.1 U/reaction uracil DNA glycosylase, primer mix and probes for *T. cruzi* and internal control, reducing agents, stabilizers and preservatives. Liquid Format.

IAC: Internal Amplification Control consisting of DNA containing non-infectious organism sequences absent in humans. Lyophilized.

PC *T cruzi*: Positive Control consisting of DNA containing specific *Trypanosoma cruzi* sequences. Lyophilized.

Nuclease-free H_2O: nuclease free water.

NON-PROVIDED REAGENTS AND EQUIPMENT

- Commercial DNA purification system (using silica columns or magnetic particles).
  Note: Although this product was validated with the High Pure PCR Template Preparation kit (Roche, Cat. 11796828001), other commercial systems may be used.
- ROX (dye that normalizes the fluorescent signal): necessary reagent for thermocyclers that require its use.
- Molecular biology quality water (nuclease free): to be used in the preparation of amplification reactions (see Complete Test Procedure).
  Note: the reconstitution of the reagents of this product is carried out with the supplied water.
- Variable volume micropipettes.
- Tips with nuclease-free filter.
- Nuclease-free microcentrifuge tubes (x 1.5 or 2 ml).
- Rack for 1.5 ml or 2 ml tubes.
- Disposable latex, vinyl, or nitrile gloves without powder.
- Reaction support according to the real-time PCR instrument used (e.g. microplates with optical films, PCR tubes, etc.).
- Thermocycler: different trademarks can be used.
  Note: This product was validated on the following thermocyclers: StepOne Plus® (Applied Biosystems), ABI7500® (Applied Biosystems) and Rotor Gene Q® (Qiagen).
- Vortex stirrer.
- Tabletop microcentrifuge with rotor for 1.5 to 2 ml tubes.
- Ice bath or cold block (for tubes x 1.5 to 2 ml).
- Container for the disposal of biological material with 5% freshly diluted sodium hypochlorite.

WARNING

- The reagents are for "in vitro" diagnostic use.
- All patient samples should be handled as if they were capable of transmitting infection.
- All components must be completely thawed (once reconstituted), homogenized and briefly centrifuged before starting the assay. It is recommended to keep them refrigerated, especially the DNA amplification mix. The latter must be smoothly homogenized, avoiding foam formation.
- Do not use the reagents after the expiration date.
- Do not interchange reagents from different lots or modify the test procedures.
- Do not use reagents of different origin than indicated.
- All reagents and samples must be discarded according to current local regulations.
- Prevent the components from undergoing microbial or nuclease contamination, when adding different elements.
- It is essential for the use of this product to have the basic knowledge in the management of molecular diagnostic techniques. Due to the high sensitivity of amplification technology, it is necessary to respect the indicated work rules for this type of analysis (sample processing areas, pre and post amplification, workflow, use of appropriate material, etc.).

STABILITY AND STORAGE INSTRUCTIONS

The kit is stable at 2-10ºC or -20ºC until the expiration date indicated on the box. The supplied *T cruzi* qPCR Master Mix UDG 2X and the lyophilized or reconstituted components are stable in refrigerator (2-10ºC) or in freezer (-20ºC). Avoid repeated freeze/thaw cycles of the Master Mix (do not thaw more than three times) in all cases, as they may cause reactivity loss. If the reagents are not used regularly, it is advisable to divide them into aliquots and freeze, taking into account the use of e nuclease-free material and that the *T cruzi* qPCR Master Mix UDG 2X contains probes that need to be protected from light.

SAMPLE

DNA from whole blood collected with EDTA anticoagulant (Do not use samples with heparin, as it has been shown to inhibit the PCR reaction).

a) Collection: in a sterile nuclease-free tube. Note: specific commercial devices can be used for this purpose.

b) Additives: EDTA as anticoagulant or solution containing guanidine as chaotropic DNA preservative.
c) Known interfering substances: high levels of hemoglobin (0.2 g/dL), triglycerides (2.8 g/dL) and conjugated bilirubin (20 mg/dL) did not interfere with the performance of the product.
d) Stability and storage instructions: Molecular assays are particularly sensitive to suboptimal preanalytical conditions, so the quality of the sample to be used is essential. It is advisable to store the sample with the specified preservative additives and at the suggested temperature until nucleic acid purification is carried out. The whole blood sample may be stored at 2-10ºC or room temperature (below 25ºC) with solutions containing guanidine (such as GE buffer (HCl-guanidine/EDTA), in 1:1 ratio with the sample). As an alternative, the whole blood-EDTA sample can also be frozen until DNA extraction.
Do not use samples with microbial contamination.
If the samples must be transported, they must be packed according to the legal specifications for the shipment of infectious material.
It is recommended that the DNA be extracted preferably with commercial systems that use magnetic columns or particles, due to its greater degree of purification and reproducibility in the results.

COMPLETE TEST PROCEDURE

1) DNA EXTRACTION from blood samples for detection of *T. cruzi* DNA

The procedure is carried out under adequate safety conditions for handling infectious material (according to NCCLS document M29-Protection of Laboratory Workers from Occupationally Acquired Infections).

Prior to DNA extraction

Before starting DNA extraction from blood samples, the following instructions must be followed:
• Disinfection of the work area: clean surfaces: countertops, pipettes, equipment, etc. 10% sodium hypochlorite (freshly diluted) or a commercial DNA decontaminant is used by spraying a paper cloth to clean micropipettes, centrifuge rotors, magnetic supports and countertops according to their respective instructions. Wash with sterile milliQ H_2O and with 70% Ethanol.
• Turn on the UV light in the work area for 15 min. Turn off UV light before using reagents to avoid damaging.
• Resuspend the IAC reagent (tube with blue cap) (see reconstitution below in 1.a_1).
• Homogenize samples and negative control with vortex for 15 sec.

1.a_1) In the "pre-amplification" area

Reconstitution of component for extraction: bring the component to room temperature (22-25ºC) for a few minutes and do a brief centrifugation to avoid losses of the lyophilized reagents when opening the tubes. Reconstitute the component with the nuclease-free water (Nuclease-free H_2O) provided, according to the following table:

Component	Reconstitution volume (µl)
IAC (blue cap)	750

Homogenize with Vortex for 30 sec. Leave 5 min at room temp. Vortex for 30 sec.
Keep the reconstituted reagent refrigerated (on ice or cold block). Store the reconstituted reagent at -20ºC or 2-10ºC, after use.

1.a_2) In the "sample processing" area

DNA extraction from whole blood - preservative

• Use a standardized extraction system that ensures a high quality of purified DNA, so a commercial DNA purification kit should be used using silica columns or magnetic particles.
• Follow the manufacturer's instructions for the DNA extraction and purification system from whole blood. After adding the lysis/extraction buffer and incubation with protease, the reconstituted IAC reagent (5 µl/sample) should be added and homogenized before loading on the column or adding the magnetic particles.
• The extracted DNA is used as a template for the amplification reaction. It must be kept until use at ≤ -20ºC, avoiding repeated freeze/thaw cycles (do not thaw more than twice). If necessary, aliquot before freezing.

2) DNA AMPLIFICATION BY REAL-TIME PCR

Before amplification

• Disinfection of the work area: clean the surfaces with a cloth dampened with 10% sodium hypochlorite (freshly diluted) or commercial DNA decontaminant: countertops, pipettes, equipment, etc.
• Turn on the UV light of the countertop and the preparation chamber of the reaction mixture for 15 min. Turn off UV light before using reagents to avoid damage.
• Thaw DNA samples and controls.
• Identify the PCR tubes according to the reaction protocol.

2.a) DNA amplification

2.a$_1$) In the "pre-amplification" area

T. cruzi/IAC reaction mixture

• Homogenize the reagents and pipette according to the following detail:

Components of the reaction mixture	Volume (µl) per reaction
T cruzi qPCR Master Mix UDG 2X	10
ROX 25 mM OPTIONAL*	0,1
Nuclease-free H_2O	4.9*/5

• Keep the reaction mixtures refrigerated (in an ice bath or cold block).
• Distribute 15 µL of the reaction mixture per tube/well of the PCR plate.
• Add 5 µL of sterile nuclease-free water (Nuclease-free H_2O) to each tube/well of the plate, corresponding to the NC (negative controls) according to the reaction protocol. Final reaction volume: 20 µl.
• Go to the "sample processing" area.

2.a$_2$) In the "sample processing" area

• Homogenize DNA samples previously extracted from whole blood with vortex and then a brief centrifugation.
• Add 5 µL of the DNA sample to each tube/well of the plate, according to the reaction protocol. Final reaction volume: 20 µl.
• Go to the "amplification/post-amplification" area.

2.a$_3$) In the "amplification/post-amplification" area

Reconstitution of the positive control for amplification: bring the component to room temperature (22-25ºC) for a few minutes and do a brief centrifugation to avoid losses of the lyophilized reagents when opening the tubes. Reconstitute the component with the Nuclease-free H_2O water provided, according to the following table:

Component	Reconstitution volume (µl)
PC *T cruzi* (red cap)	500

Homogenize with Vortex for 30 sec. Let stand for 5 min at room temp. Vortex for 30 sec.
• Keep the reconstituted reagent refrigerated (on ice or cold block). Store the reconstituted reagent at -20ºC or 2-10ºC, after use.

2.a$_4$) Preparation of the strong and weak positive controls ("amplification/post-amplification" area)

Note: sit is recommended to manipulate the PC *T cruzi* reagent in the post-amplification area, to avoid contaminating the other reagents.
1) Add 450 µl of nuclease-free water in 4 tubes labeled 1 to 4.
2) Add 50 µl of PC *T cruzi* reagent (red cap) in tube 1.
3) Shake with vortex to ensure good homogenization.
4) Change the tip of the micropipette and add 50 µl of the mixture from tube 1 to tube 2: (High PC *T cruzi*)
5) Shake with vortex to ensure good homogenization.
6) Repeat steps 4 and 5 until the dilution series is complete (tube 4 (Low PC *T cruzi*))

Standard curve	Dilution	eq parasites/ml
PC *T cruzi* reagent (red cap)	-	1000
Tube 1	1/10	100
Tube 2 (High PC *T cruzi*)	1/100	10
Tube 3	1/1,000	1
Tube 4 (Low PC *T cruzi*)	1/10,000	0.1

The concentration of High PC *T cruzi* is equal to 10 parasites/ml blood and the concentration of Low PC *T cruzi* is equal to 0.1 parasites/ml blood.

2.a$_5$) Reaction conditions

• Program the thermocycling profile on the equipment:

Stage	Time	Temperature	
UDG Incubation*	2 min	50ºC	
Enzymatic activation	10 min	95ºC	
Denaturation	15 seg	95ºC	45 cycles
Data collection**	60 seg	58ºC	

* Incubation with UDG (Uracil-DNA Glycosylase) prevents carryover amplification prior to the specific amplification reaction.
** Fluorescence data is taken on the FAM and VIC channels in reaction wells/tubes where DNA and NC are quenched. In the rest of the wells (Positive controls) they are taken only in the FAM channel.

- Add 5 µL sample of the positive controls High PC *T cruzi* (dilution 1/100) and Low PC *T cruzi* (dilution 1/10,000) to the tubes/wells of the corresponding plate, according to the reaction protocol. Final reaction volume: 20 µl.
- Close the tubes or seal with the corresponding film on the plate.
- Introduce the tubes or plate in the real time PCR equipment.
- Start the reaction.

INTERPRETATION OF RESULTS

Assay Validation Criteria

To carry out the analysis of the results obtained during the amplification, it must be previously assessed whether the parameters mentioned below are correctly set automatically by the instrument (default) or if necessary fix them manually:

- Baseline (bottom signal of all microplate wells): it is recommended that it ends (baseline end) 2 or 3 c Cycles below the lowest Ct value (cycle above the fluorescence threshold) of the amplification curves.
- Threshold line (fluorescence threshold value): must be set in the exponential phase of the amplification curves. It is recommended to set its value to 10% of the fluorescence of the general plateau.

Once the above parameters are defined, the test is considered valid if the following conditions are simultaneously met:

1- The NCs must not present amplification curves and in case of any curve that exceeds the Threshold Line, the Ct value must be ≥ 39. Otherwise, it indicates random or component contamination. In the random contamination that occurs during the loading of DNA into the reaction mixture, some or all of the NCs are contaminated showing amplification curves with different CT values. In the contamination of one or more reagents, all NCs are contaminated, showing curves with similar CT values.

2- The High PC *T cruzi* corresponding to tube 2 should have a Ct value between 24.5 and 30.5 (this depends on the thermocycler used). This range is for reference and may be slightly different in non-validated equipment, so the user is recommended to establish their own range and acceptance criteria.

3- The Low PC *T cruzi* corresponding to tube 4 should have a Ct value between 31 and 37 (this depends on the thermocycler used). This range is for reference and may be slightly different in non-validated equipment, so the user is recommended to establish their own range and acceptance criteria.

4- A difference between both positive controls must be maintained between 6 and 8 CT units.

5- IAC analysis: the expected Ct value and signal for the amplification of the internal control in a given sample, vary according to the number of copies of the *T. cruzi* genome, to the efficiency of the DNA purification process, to the thermocycler used in the reaction, etc. Ct ≤ 28 values are within the expected range for the validated DNA purification system (set according to the purification system to be used). It can happen with samples of high parasite load, that the internal control does not amplify or show Ct values > 28 or the established value, due to the competence in the consumption of the reagents involved, in any case this does not invalidate the result.

If any of the conditions obtained does not meet the specifications, omit the wells that may generate deviations from the analysis and/or analyze with the appropriate criteria the need or not to repeat the test. Bear in mind that the defined conditions are those of an optimal test and that values close to those described can be accepted, analyzing them together.

Analysis and interpretation of the results

The integrated results can be interpreted as follows:

- SIt is considered a positive sample, detectable for *T. cruzi*, when the sigmoid fluorescence curve crosses the threshold (Threshold line) yielding a value of Ct ≤ 36.
- It is considered a negative sample, not detectable for *T. cruzi*, when the sigmoid fluorescence curve does not cross the threshold resulting in the absence of Ct or crosses it with a value greater than 39, and in addition, the internal control presents an amplification curve with CT ≤ 28 values.
- It is considered a doubtful sample when the sigmoid fluorescence curve crosses the threshold yielding a Ct value between 36 and 39. Because it is the area where there may be random cross-contamination or reagents by manipulation, it is recommended to repeat the extraction from the sample, and if not possible, amplify from the extracted DNA.

DNA quenching (*T cruzi*)	Internal Control (IAC)	Negative Control	High PC *T cruzi*	Low PC *T cruzi*	Interpretation
+	+/-	-	+	+	*T. cruzi* DNA presence
-	+	-	+	+	*T. cruzi* DNA not detected
+/-	+	-	+	-	Low sensitivity assay. Repeat
-	-	-	-	-	Invalid result
+	+	+	+	+	Invalid result
+	-	+	+	+	Invalid result

PRODUCT SPECIFICATIONS

Product validation tests were carried out within the framework of the project to develop an early diagnosis system for Chagas disease, carried out from the consolidation of a public-private associative consortium made up of two national scientific institutions (the Laboratory of Molecular Biology of Chagas Disease from the Institute of Research in Genetic Engineering and Molecular Biology (LaBMECh-INGEBI-CONICET) and the National Institute of Parasitology Dr. Mario Fatala Chabén (INP, dependent on ANLIS) and Wiener Laboratorios S.A.I.C.

a) Analytical Sensitivity:

This product has a Positive Limit of Detection (LOD) of 95% (C_{95}) of 0.148 p/ml, calculated by Probit analysis.

b) Analytical Specificity:

The ***T. cruzi* DNA test** does not show cross reactivity with DNA sequences from other parasites such as *Trypanosoma rangeli* and Leishmania species that coexist in the same endemic region of *Trypanosoma cruzi* and can cause coinfection in some patients.

c) Precision:

The inter and intra-assay precision of the ***T. cruzi* DNA test** was studied for the entire process (from DNA extraction to amplification) following the recommendations of the CLSI Guideline EP15-A2. Three levels of parasite load in the area close to the LOD (100 pairs/100 ml, 50 pairs/100 ml and 25 pairs/100 ml) were tested using 40 replicates of each level, and it was statistically analyzed.

Log_{10} Nominal concentration of *T. cruzi*	Nominal value	S.D. intra-assay	C.V. (%) intra-assay	Total S.D.	Total C.V. (%)
Log_{10} 100 par/100 ml	2	0.27	12.47	0.356	16.45
Log_{10} 50 par/100 ml	1.69897	0.38	21.53	0.492	27.88
Log_{10} 25 par/100 ml	1.39794	0.666	44.1	0.707	46.86

The product shows intra-assay and total deviations in the quantification limit area of 12.47-21.53% and 16.45-27.88%, respectively.

d) Inclusivity:

The ***T. cruzi* DNA test** detects all the lineages or discrete typing units (DTUs) of *Trypanosoma cruzi* that infect humans (Tc Ia, Tc Id, Tc Ie, Tc II, Tc III, Tc IV, Tc V and Tc VI).

Detection of all relevant DTUs of *T. cruzi* infection in humans is guaranteed with the design of the primer sequences and the ***T. cruzi* DNA test** product probe. These sequences are 100% homologous with a wide range of clinically relevant reference sequences, based on comprehensive bioinformatic analysis.

e) Linear Range:

To determine the Linearity of ***T. cruzi* DNA test**, 9 concentration levels were analyzed in quadruplicate, covering the clinical

range (12.5-10000000 *T. cruzi* parasites/100 ml whole blood) following the recommendations of the EP6-A Guideline from CLSI. The product showed a linear response between 50-10^2 (log = 1.7-2) and at least 10^7 (log = 7) *T. cruzi* parasites/100 ml whole blood, as observed in Figure 1. It can also be expressed as a linear range between 0.5-1 and 10^5 parasites/ml.

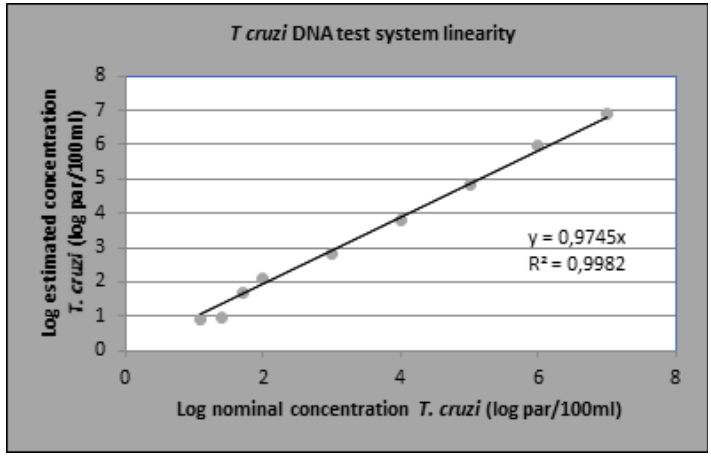

Figure 1: Linearity of ***T. cruzi*** **DNA test**

f) Clinical Sensitivity and Specificity:

Population of infants and children with congenital Chagas

For clinical validation, a prospective population study with blood samples from babies born to infected mothers, masked, collected from public institutions in Argentina, was proposed. Patients were followed at birth, 1-2 months and at 10 months of age, comparing them with the gold standard method for early diagnosis (microhematocrit - MH) in the framework of the algorithm in force in the country's Chagas health system. This work was carried out by the Institute of Clinical and Sanitary Effectiveness (IECS) supervised by the National Institute of Parasitology Mario Fatala Chaben (INP) and the Institute of Research in Genetic Engineering and Molecular Biology (INGEBI).

Study sites: The study was carried out with samples from the Julio Perrando Hospital in Resistencia (Chaco) and the National Institute of Parasitology "Dr. Mario Fatala Chaben "(INP) from CABA (Buenos Aires) who enrolled children from the province after birth who were treated for control.

265 patients were enrolled, and 165 cases were selected for the final comparative analysis. There were 11 children with congenital infection. Of the 11 cases with vertical transmission infection, 5 cases were detected by microhematocrit within 2 months after birth and the other 6 cases only at 10 months by serology (ELISA, IHA or IIF). The real time PCR developed detected 9 of the congenital cases within 2 months of life.

The parameters of sensitivity and clinical specificity of both methods were as follows:

Clinical sensitivity ***T. cruzi*** **DNA test**: 81.82%
MH clinical sensitivity: 45,45%
Clinical specificity ***T. cruzi*** **DNA test**: 98.7%
MH clinical specificity: 100%

g) Correlation Study with other methods:

The study was carried out in a population of samples from chronic and congenital patients with Chagas disease. The corresponding DNA were processed and analyzed with the ***T. cruzi*** **DNA test** and with another similar system of real time PCR. These DNAs were extracted from frozen or guanidine-buffered EDTA-blood from chronic (60 samples), congenital (4) and negative (7) patients. The method that was used as reference system in the analysis was FastStart Universal Probe Master (ROX) commercial Master Mix (Cat: 04913949001) from Roche, used in different studies for molecular diagnosis of Chagas (Schijman et al 2011, Duffy et al 2013, Cura et al 2015, Ramirez et al 2015, Martinez et al 2016, Ramirez et al 2017).

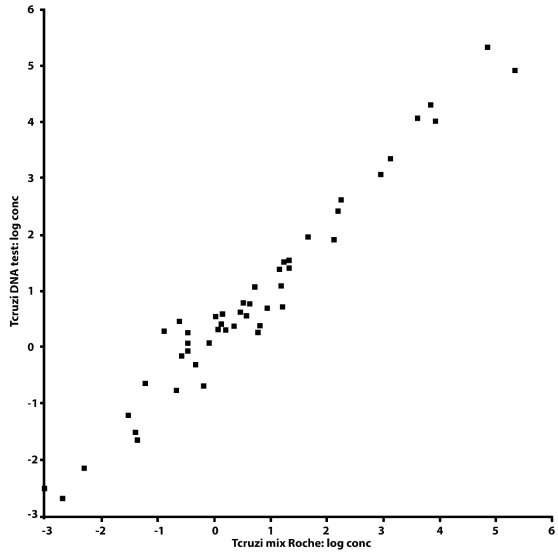

Figure 2: Correlation study between methods

The correlation coefficient r is 0.98.
The correlation analysis of logarithm values of parasite concentration/ml of the samples detected with the ***T. cruzi*** **DNA test**, show a high correlation (r = 0.98) with the evaluated system (Figure 2).

SIZE
Kit for 48 tests (Cat. Nº 1060010):
2 x 300 uL T cruzi qPCR Master Mix UDG 2X
1 x → 500 uL PC T cruzi
1 x → 750 uL IAC
1 x 2 mL Nuclease-free H_2O

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Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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