External Quality Control Program for Real-Time PCR Testing in a Multi-Centre, Randomised Controlled Clinical Trial in Chagas Disease

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BACKGROUND

✓ Accurate diagnostic tools as well as markers of parasitological response to treatment, are priorities in Chagas disease research and development.

✓ Real-Time PCR (qPCR) testing is an increasingly recommended diagnostic endpoint in clinical trials of drug candidates in Chagas disease.

✓ Recent studies have assessed performance characteristics of qPCR methods, but no formal external quality control program has provided performance assessment of the assays in use in clinical trials.

AIM

This study aimed to characterize the performance of the qPCR method as a diagnostic endpoint in the E1224 clinical trial through the analysis of an external quality control specially devised forthis purpose.

MATERIALS AND METHODS

Table 1. Design of Quality Control Panels for E1224 trial.

T. cruzi stocks	Concentration (par. eq./mL)	QC Panel I (day 0)	QC Panel II (3 months)	QC Panel III (6 months)	QC Panel IV (9 months)
TcIa K98	0	CCP 101	CCP 208	CCP 306	CCP 401
	1	CCP 102	CCP 206	CCP 305	CCP 403
	10	CCP 103	CCP 207	CCP 307	CCP 402
	100	CCP 104	CCP 205	CCP 308	CCP 404
TcId Sylvio X10 Cl1	0	CCP 107	CCP 201	CCP 301	CCP 407
	1	CCP 106	CCP 202	CCP 303	CCP 406
	10	CCP 105	CCP 204	CCP 302	CCP 405
	100	CCP 108	CCP 203	CCP 304	CCP 408
TcV LL014-1-R1 Cl1	0	CCP 109	CCP 213	CCP 313	CCP 409
	1	CCP 110	CCP 214	CCP 316	CCP 410
	10	CCP 112	CCP 215	CCP 315	CCP 412
	100	CCP 111	CCP 216	CCP 314	CCP 411
TcVI CL-Brener	0	CCP 116	CCP 209	CCP 311	CCP 416
	1	CCP 114	CCP 211	CCP 310	CCP 414
	10	CCP 115	CCP 210	CCP 309	CCP 415
	100	CCP 113	CCP 212	CCP 312	CCP 413

DNA extraction:
300 µL of Guanidine-EDTA-Blood samples spiked with 200 pg of an Internal Amplification Control (IAC) were processed using of High Pure PCR Template Preparation kit (Roche, USA) and eluted in 100 µL of elution buffer.

qPCR procedure:
Duplex qPCR targets the satellite *T. cruzi* DNA and the IAC DNA sequences. The qPCR reactions were carried out using FastStart Universal Probe Master Mix (Roche, Germany) with 5 µL of DNA in a final volume of 20 µL.

Cycling: 10 minutes at 95 ºC
40 cycles: 15 seconds at 95 ºC
1 minute at 58 ºC

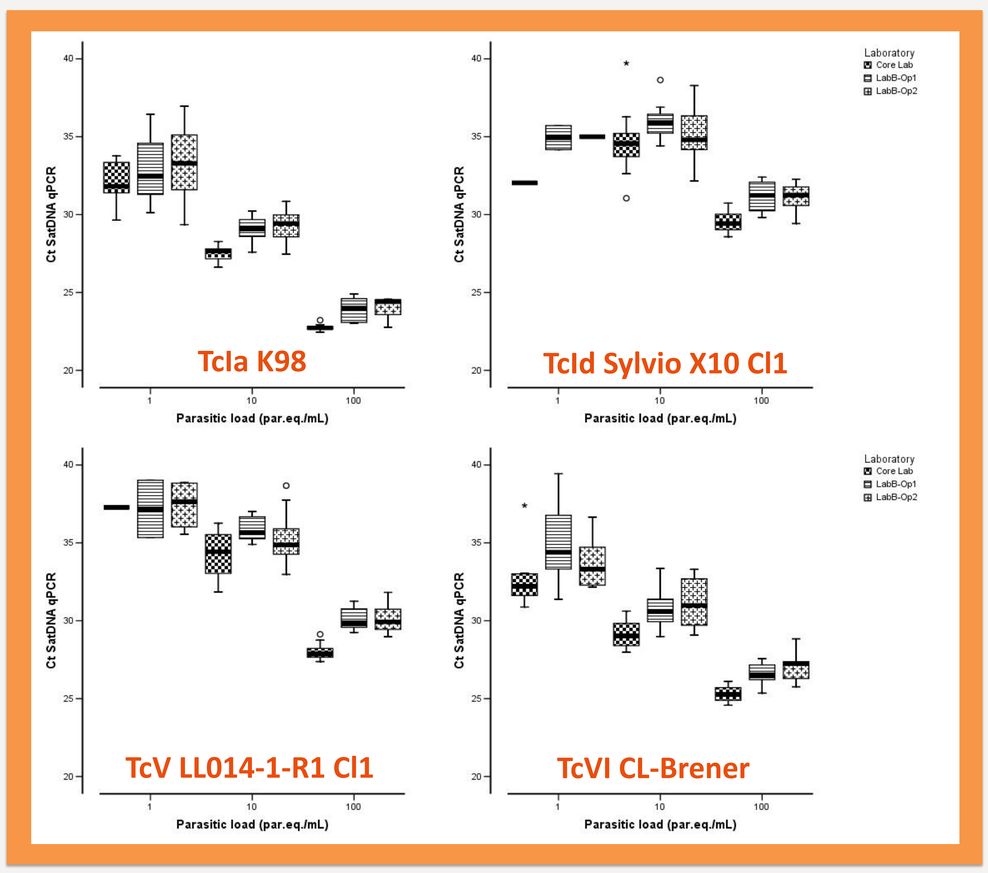
qPCR devices:
ABI7500 (Applied Biosystems, USA) >>> Core Lab
Rotor-Gene Q (Corbett Life Science, UK) >>> LabB

RESULTS

Table 1. Accordance and Concordance analysis of qualitative qPCR results.

T. cruzi stocks	Laboratory	Total of Replicates	Number of Positive Replicates 1 par. eq./mL	10 par. eq./mL	100 par. eq./mL
All	Core Lab	44	22	43	44
	LabB-Op1	48	26	42	48
	LabB-Op2	48	29	45	48
	Accordance [CI95] (%)		49.8 [49.1-54.3]	86.6 [79.7-94.3]	100 [100-100]
	Concordance [CI95] (%)		50.3 [48.6-53.1]	86.7 [79.0-94.4]	100 [100-100]
	COR [CI95]		0.98 [0.96-1.18]	0.99 [0.97-1.13]	1 [1-1]
TcIa K98	Core Lab	11	11	11	11
	LabB-Op1	12	12	12	12
	LabB-Op2	12	12	12	12
	Accordance [CI95] (%)		100 [100-100]	100 [100-100]	100 [100-100]
	Concordance [CI95] (%)		100 [100-100]	100 [100-100]	100 [100-100]
	COR [CI95]		1 [1-1]	1 [1-1]	1 [1-1]
TcId Sylvio X10 Cl1	Core Lab	11	1	10	11
	LabB-Op1	12	2	8	12
	LabB-Op2	12	1	11	12
	Accordance [CI95] (%)		78.1 [62.6-94.7]	71.7 [60.4-89.3]	100 [100-100]
	Concordance [CI95] (%)		79.7 [63.2-94.4]	70.3 [52.1-89.1]	100 [100-100]
	COR [CI95]		0.91 [0.89-1.41]	1.07 [0.90-2.40]	1 [1-1]
TcV LL014-1-R1 Cl1	Core Lab	11	1	11	11
	LabB-Op1	12	2	10	12
	LabB-Op2	12	4	10	12
	Accordance [CI95] (%)		66.8 [56.2-88.2]	78.6 [66.8-94.1]	100 [100-100]
	Concordance [CI95] (%)		67.2 [52.7-88.7]	79.4 [62.3-94.4]	100 [100-100]
	COR [CI95]		0.99 [0.89-2.14]	0.95 [0.91-1.41]	1 [1-1]
TcVI CL-Brener	Core Lab	11	9	11	11
	LabB-Op1	12	10	12	12
	LabB-Op2	12	12	12	12
	Accordance [CI95] (%)		79.7 [70.1-94.7]	100 [100-100]	100 [100-100]
	Concordance [CI95] (%)		78.9 [61.9-94.4]	100 [100-100]	100 [100-100]
	COR [CI95]		1.05 [0.98-1.71]	1 [1-1]	1 [1-1]

Accordance: Intra-laboratory agreement
Concordance: Inter-laboratory agreement
COR: Concordance Odds Ratio
COR= Accordance * (100 - Concordance) / Concordance * (100 - Accordance)



There were statistical differences between both laboratories but not between both operators from LabB for all *T. cruzi* stocks.

Figure 1. Analysis of inter-laboratory precision for all *T. cruzi* stocks.

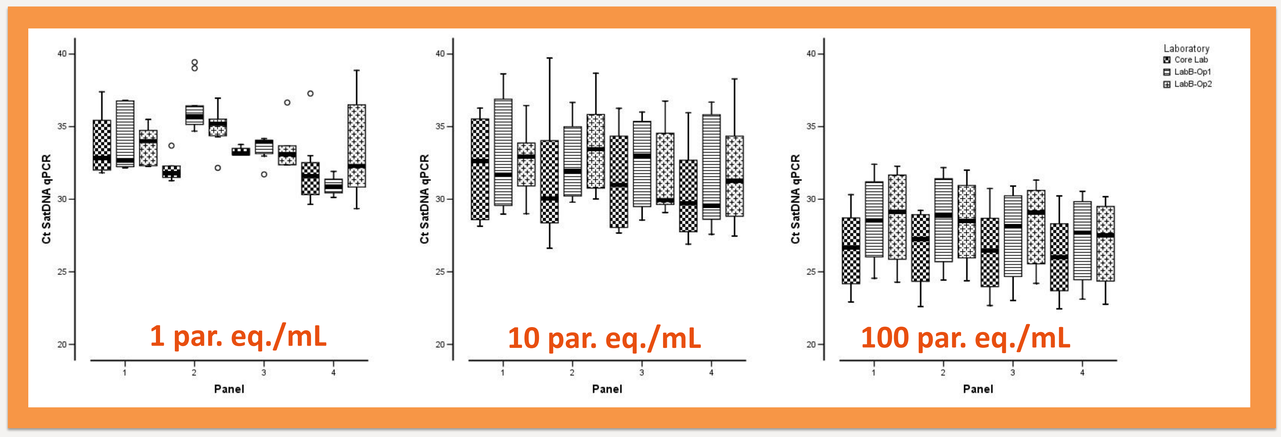


Figure 2. Analysis of QC Panels' stability after 9 months of preparation.

CONCLUSIONS

✓ There was a high within (Accordance) and between (Concordance) laboratory agreement in the qualitative results of this study, independently of the *T. cruzi* DTU and stock used.

✓ The results obtained for the QC panels after nine months of preparation evidenced the high stability of Guanidine-EDTA-blood samples (no statistical differences between panels).

✓ An External Quality Control Program for molecular diagnosis of Chagas disease is feasible and informative, allowing broader implementation of qPCR testing in clinical trial settings.

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