

Rapid, Colorimetric Detection of *Trypanosoma cruzi* via Loop-Mediated Isothermal Amplification

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Supplementary Data

Table S1: LAMP primers targeting the nuclear satellite region of <i>T. cruzi</i>	2
Table S2: qPCR primers and probe targeting the nuclear satellite region of <i>T. cruzi</i>	2
Figure S1: <i>T. cruzi</i> LAMP assay LOD using strain Dm28c (Tcl)	3
Figure S2: <i>T. cruzi</i> LAMP assay LOD using strain G (Tcl).....	3
Figure S3: <i>T. cruzi</i> LAMP assay LOD using strain CL (TcVI)	4
Figure S4: <i>T. cruzi</i> LAMP assay selectivity.....	4
Figure S5: Detection of <i>T. cruzi</i> DNA extracted with modified NucliSENS easyMAG Kit....	5
Figure S6: Detection of <i>T. cruzi</i> DNA extracted with MagMAX Kit.....	5
Figure S7: Identification of <i>T. cruzi</i> from canine blood samples – group 1	6
Figure S8: Identification of <i>T. cruzi</i> from canine blood samples – group 2	7

Table S1: LAMP primers targeting the nuclear satellite region of *T. cruzi*. Borrowed from Ordonez *et al.*

Primer	Sequence (5' to 3')
F3	AACTATCCGCTGCTTGGA
B3	AAGAGCTCGCGAAATTCC
FIP	CCCACCATTCAACAATCGGAAACCACTCGGCTGATCGTTTT
BIP	AGTCAGAGGCACTCTCTGTCAACCAAGCAGCGGATAGTTC
LF	TTGGACCACAACGTGTGAT
LB	TTCACACACTGGACACCAAA

Table S2: qPCR primers and probe targeting the nuclear satellite region of *T. cruzi*. Borrowed from Duffy *et al.*

Primer	Sequence (5' to 3')
Forward	ACTCGGCTGATCGTTTTCGA
Backward	AATTCCTCCAAGCAGCGGATA
Probe	FAM-CACACACTGGACACCAA-NFQ-MGB

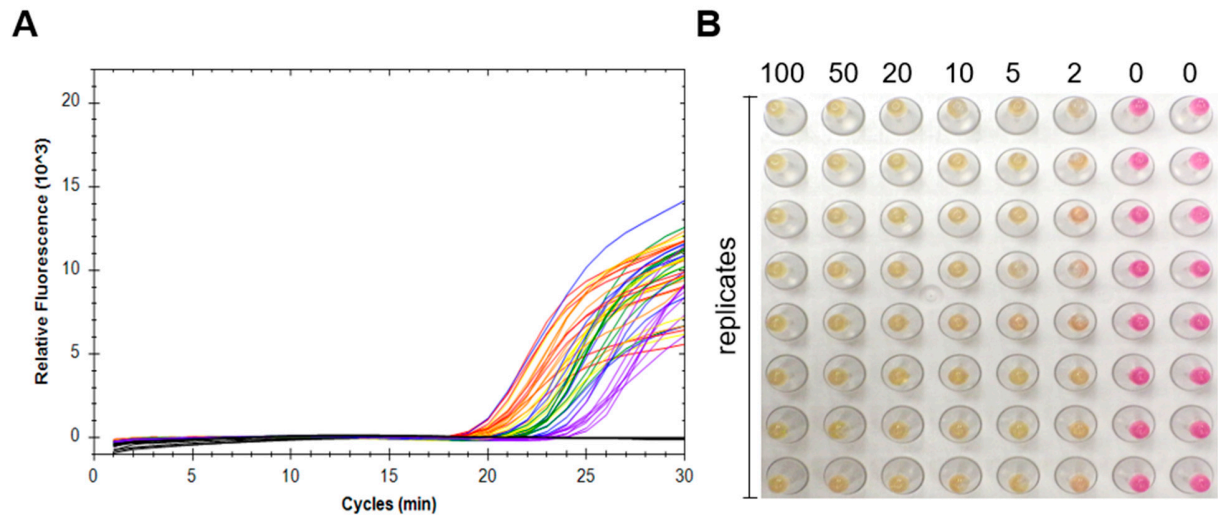


Figure S1: *T. cruzi* LAMP assay LOD using strain Dm28c (Tcl). (A) Real-time amplification curves and (B) end-point colorimetric results with 8 replicates at each concentration (16 for negative control). Concentrations tested were 100 (red), 50 (orange), 20 (yellow), 10 (green), 5 (blue), 2 (purple), and 0 (black) genome copies/reaction.

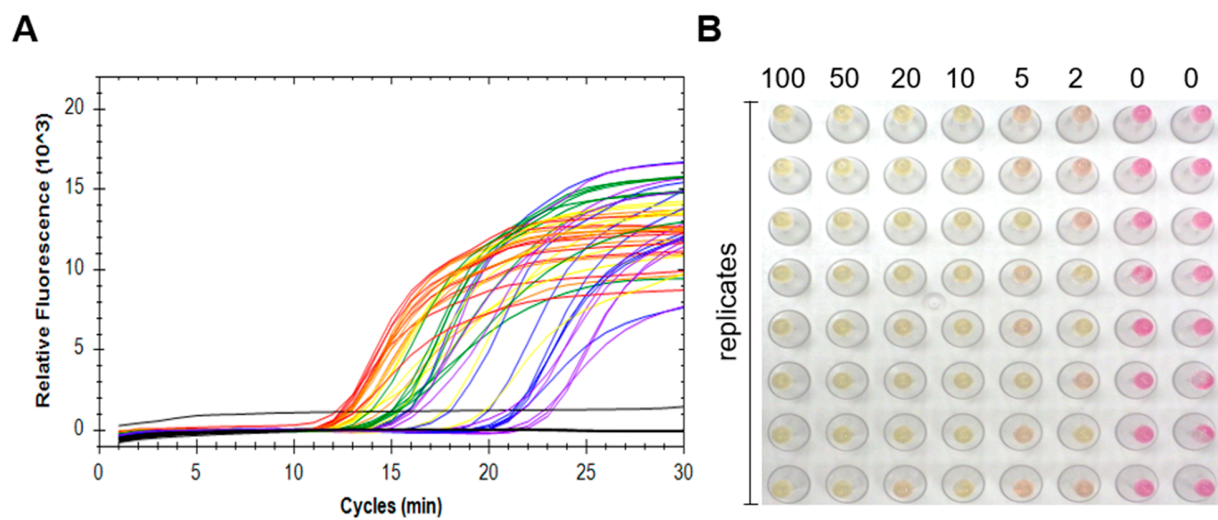


Figure S2: *T. cruzi* LAMP assay LOD using strain G (Tcl). (A) Real-time amplification curves and (B) end-point colorimetric results with 8 replicates at each concentration (16 for negative control). Concentrations tested were 100 (red), 50 (orange), 20 (yellow), 10 (green), 5 (blue), 2 (purple), and 0 (black) genome copies/reaction.

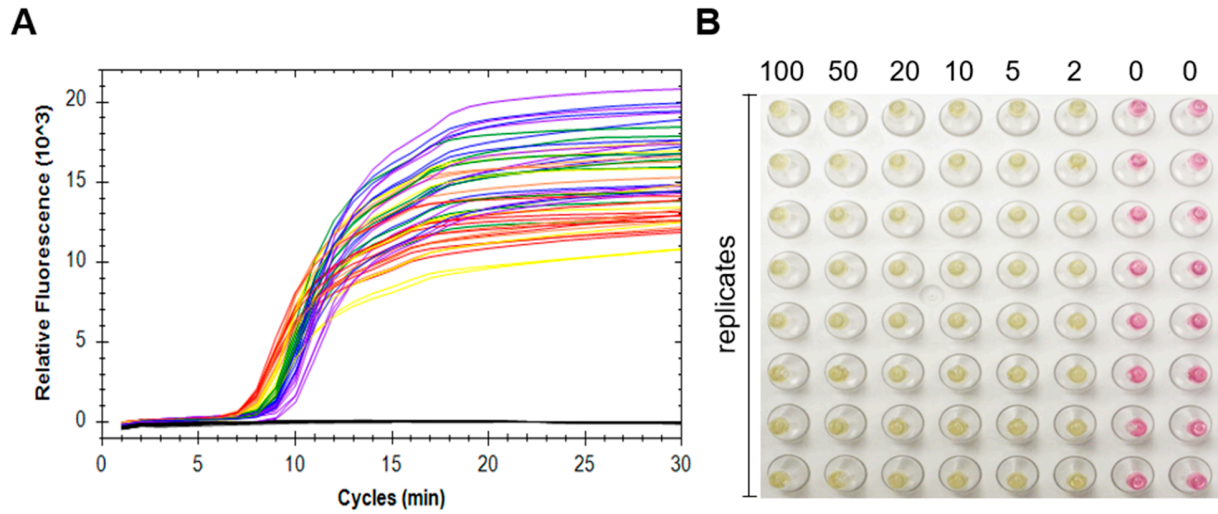


Figure S3: *T. cruzi* LAMP assay LOD using strain CL (TcVI). (A) Real-time amplification curves and (B) end-point colorimetric results with 8 replicates at each concentration (16 for negative control). Concentrations tested were 100 (red), 50 (orange), 20 (yellow), 10 (green), 5 (blue), 2 (purple), and 0 (black) genome copies/reaction.

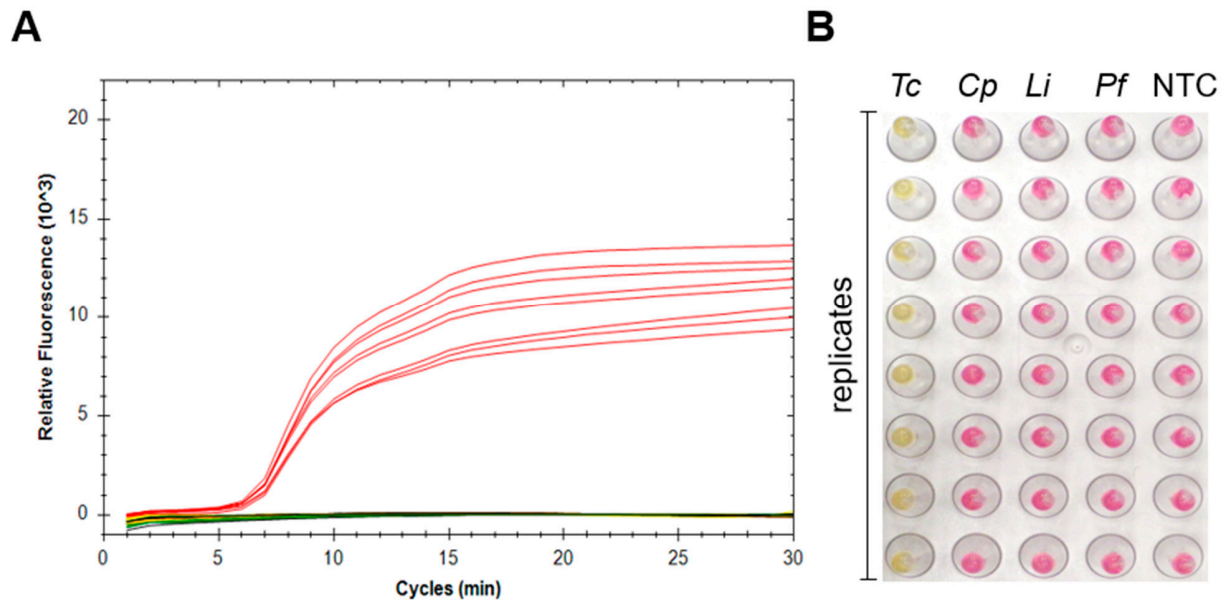


Figure S4: *T. cruzi* LAMP assay selectivity. (A) Real-time amplification curves and (B) end-point colorimetric results with 8 replicates for each sample. *T. cruzi* (red) samples amplified in real time and showed a clear color change at end point in comparison to *C. parvum* (orange), *L. infantum* (yellow), *P. falciparum* (green), and NTC (black) samples. All samples were tested at 500 genome copies/reaction.

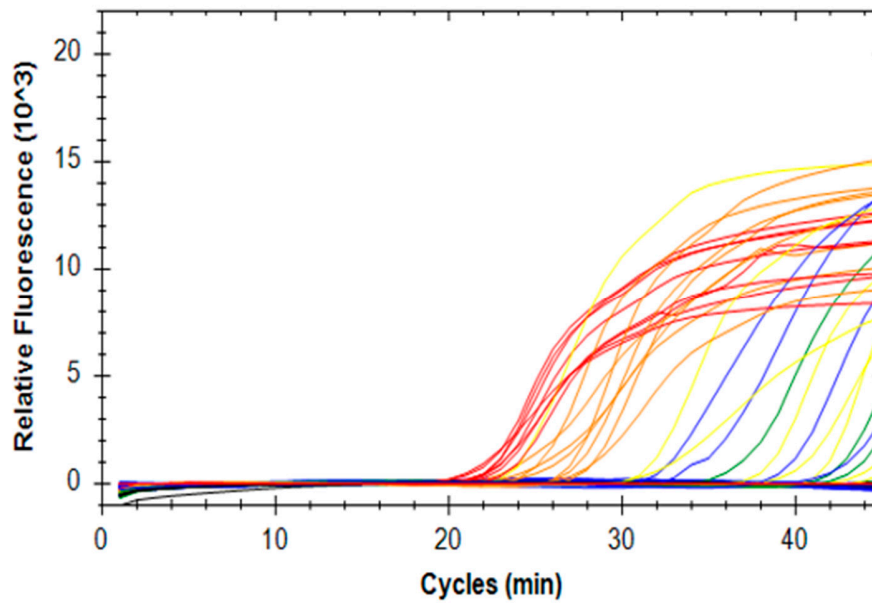


Figure S5: Detection of *T. cruzi* DNA extracted with modified NucliSENS easyMAG Kit. *T. cruzi* parasites were spiked into human whole blood prior to extraction and then used at 120 (red), 12 (orange), 1.2 (yellow), 0.12 (green), 0.012 (blue), and 0 (black) parasite equivalents/reaction. Real-time curves show amplification at higher concentrations.

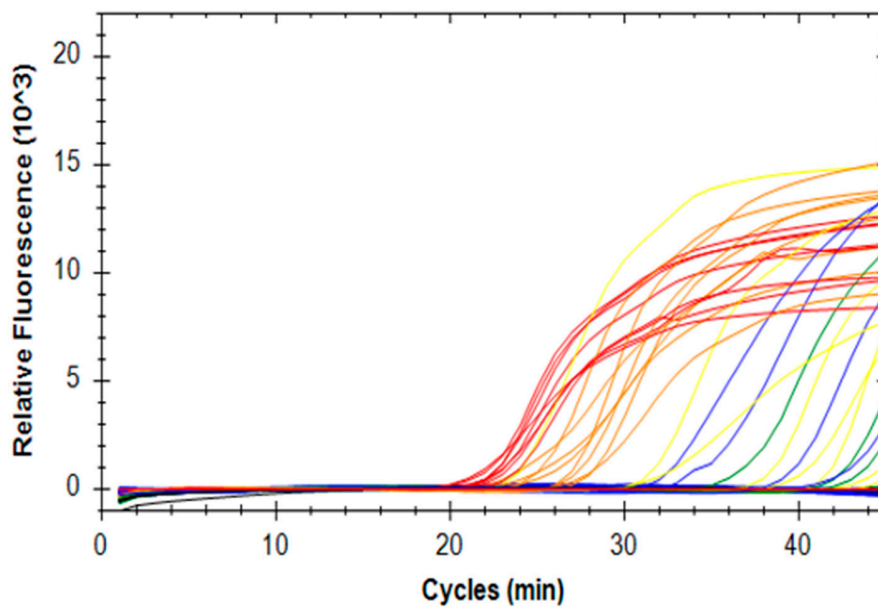


Figure S6: Detection of *T. cruzi* DNA extracted with MagMAX Kit. *T. cruzi* parasites were spiked into human whole blood prior to extraction and then used at 120 (red), 12 (orange), 1.2 (yellow), 0.12 (green), 0.012 (blue), and 0 (black) parasite equivalents/reaction. Real-time curves show amplification at higher concentrations.

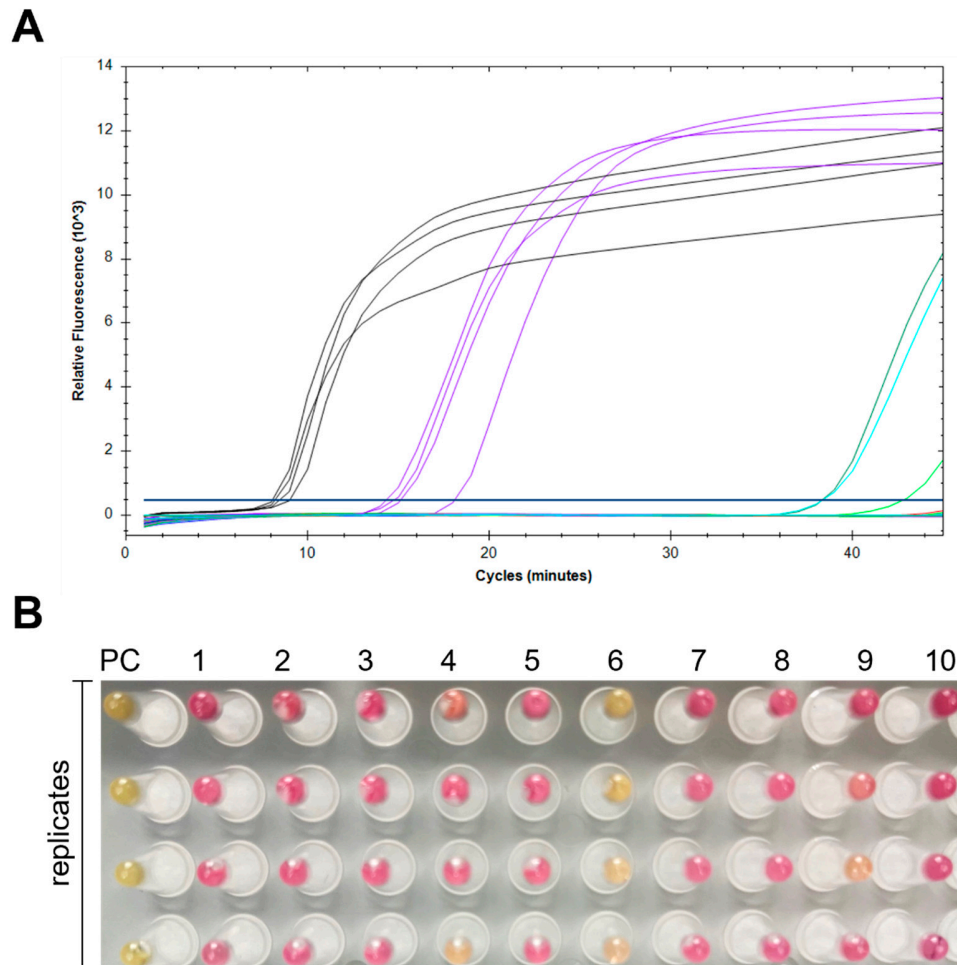


Figure S7: Identification of *T. cruzi* from canine blood samples – group 1. qPCR results were blinded from the researcher performing LAMP. (A) Real-time amplification curves and (B) end-point colorimetric results with 4 replicates at each concentration. Results represent 1 of 2 repeats. Samples were labeled as positive control (PC) (black), 1 (red), 2 (orange), 3 (yellow), 4 (green), 5 (blue), 6 (purple), 7 (pink), 8 (lime), 9 (turquoise), and 10 (lavender).

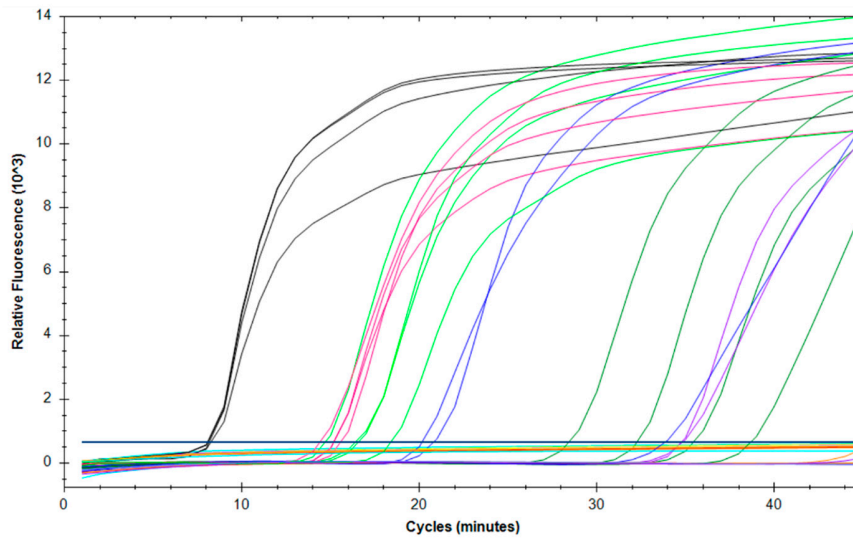
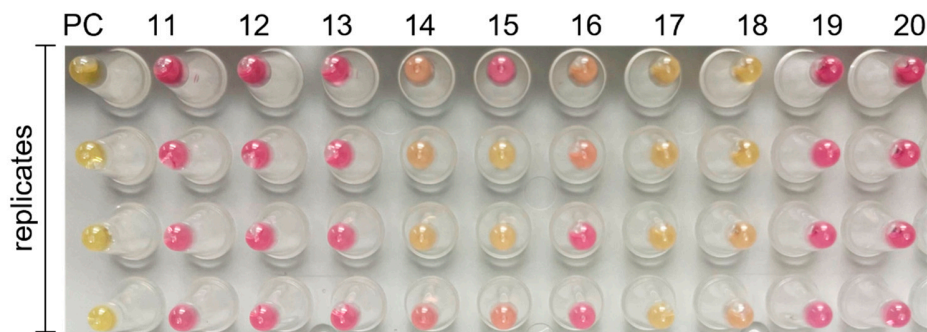
A**B**

Figure S8: Identification of *T. cruzi* from canine blood samples – group 2. qPCR results were blinded from the researcher performing LAMP. (A) Real-time amplification curves and (B) end-point colorimetric results with 4 replicates at each concentration. Results represent 1 of 2 repeats. Samples were labeled as positive control (PC) (black), 11 (red), 12 (orange), 13 (yellow), 14 (green), 15 (blue), 16 (purple), 17 (pink), 18 (lime), 19 (turquoise), and 20 (lavender).