Mundo Sano

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Evaluation of the performance of a LAMP prototype kit for Chagas detection in clinical samples

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INTRODUCTION

Chagas Disease - American Trypanosomiasis

- Caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*).
- About 7 million people affected worldwide
- Transmission types of Chagas disease:
  - Vectorial:
    - triatomine insect vector

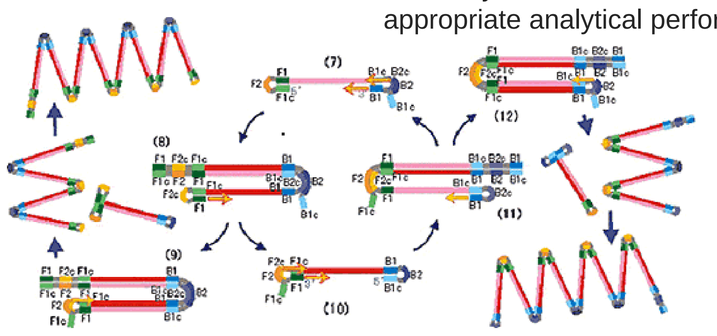
Triatoma infestans

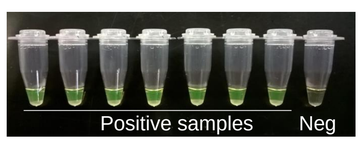
Trypanosoma cruzi

  - Non vectorial:
    - congenital transmission
    - blood transfusion or organ transplantation from infected donors
    - oral transmission by consuming contaminated food

-Diagnosis:
- Microscopy: only for acute infections, requires qualified personnel
- Serology: "gold standard" diagnostic technique for chronic stage, not suitable for Chagas detection in newborns
- Molecular methods:
  - **qPCR** (real time PCR):
    - high sensitivity and specificity
    - requires a thermocycler and qualified personnel
  - **LAMP** (Loop mediated isothermal amplification):
    - novel and rapid method (40 minutes)
    - *Bst* DNA polymerase enables amplification under isothermal conditions

-high specificity because of the use of four primers recognizing six distinct regions on the target
- requires only a water bath at 65ºC
- detection by naked eye
- recently evaluated for its use in Chagas diagnosis, showing an appropriate analytical performance (Besuschio *et al.* 2017)





AIM OF THIS STUDY

Evaluation of sensitivity and specificity of LAMP compared to standardized qPCR in different clinical groups: acute, congenital, chronic and reactivated infection and a sero-negative control group.

METHODS

Samples: blood-EDTA samples of patients of different clinical groups:
- Sero-negative control group
- Acute infection
- Congenital infection
- Chronic infection
- Reactivated infection

Written informed consents were obtained for all patients

DNA extraction: commercial kit (Roche)

qPCR:
T. cruzi satellite DNA with IAC (internal amplification control) (Duffy *et al.* 2013):
Primer Cruzi1 5´-ASTCGGCTGATCGTTTTCGA-3´ (0.75µM)
Primer Cruzi2 5´ -AATTCCTCCAAGCAGCGGATA-3´ (0.75µM)
Probe Cruzi3 5´ -Fam-CACACACTGGACACCAA-NFQ-MGB-3´ (0.05µM)
Primer IAC IAC Fw 5´ -ACCGTCATGGAACAGCACGTA-3´ (0.1µM)
Primer IAC Rv 5´ -CTCCCGCAACAAACCCTATAAAT-3´ (0.1µM)
Probe IAC Tq 5´ -VIC-AGCATCTGTTCTTGAAGGT-NFQ-MGB-3´ (0.05µM)
DNA sample: 5 µl
Cycling conditions:
- 95ºC 10 min
- 95ºC, 15 sec
- 58ºC, 1 min

] 40 cycles

LAMP:
LAMP prototype kit includes:
- *Bst* DNA polymerase
- Deoxynucleotide triphosphates
- Magnesium sulfate
- Calcein
- Manganese chloride
- Primers

DNA sample: 30 µl
Incubation: 40 minutes, 65ºC

RESULTS

Sensitivity = Ssv = TP/ (TP +FN) x100

Specificity = Spf= TN/(FP+TN) x100

True Negative = TN
True Positive = TP
False Negative = FN
False Positive = FP
CI = Confidence Interval 95%

Overall Values

Ref.test / Index test	qPCR positive	qPCR Negative	Total	Parameters (CI)
LAMP positive	TP = 38	FP = 3	41	Ssv = 88.4 (78.7 -93.3)
LAMP negative	FN = 5	TN = 127	132	Spf = 97.7 (94.5 -99.3)
Total	43	130	173	-------

Clinical group	Number of patients
Sero-negative control group	21 Children 67 Adults
Acute infection	3
Congenital infection	10
Chronic infection	43
Reactivated infection	23
Total	173

Acute **infection**

Ref.test / Index test	qPCR positive	qPCR Negative	Parameters (CI)
LAMP positive	TP = 2	FP = 0	Sample size not fit for calculation
LAMP negative	FN = 0	TN = 1	Sample size not fit for calculation

Congenital **Infection**

Ref.test / Index test	qPCR positive	qPCR Negative	Parameters (CI)
LAMP positive	TP = 12	FP = 0	Ssv = 75% (56.7-75.0)
LAMP negative	FN = 4	TN = 21	Spf = 100% (86.1 -91.3)

Chronic **Infection**

Ref.test / Index test	qPCR positive	qPCR Negative	Parameters (CI)
LAMP positive	TP = 7	FP = 3	Ssv = 100% (61.3-100)
LAMP negative	FN = 0	TN = 100	Spf = 97.1% (94.5-97.1)

Reactivated infection

Ref.test / Index test	qPCR positive	qPCR Negative	Parameters (CI)
LAMP positive	TP = 17	FP = 0	Ssv = 94.4 % (81.6 -94.4)
LAMP negative	FN = 1	TN = 5	Spf = 100% (53.4 -100)

For each clinical group the accuracy parameters for LAMP are referred to qPCR as the reference test but not to the clinical gold standard.

CONCLUSION

We obtained high overall values of specificity and sensitivity and little differences between LAMP and qPCR.

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