Aug 29, 2025 Version 2

Standardization of the technique 'LOOP-MEDIATED ISOTHERMAL AMPLIFICATION' (LAMP) using the target 18S rDNA for cutaneous leishmaniasis V.2

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvw4dj9lmk/v2

Elizabeth Cristina Araujo Ferreira Faulhaber[1], Daniel Moreira Avelar[2], Gabrielle Baldan Stanciola[1], Luanna da Silva Ventura[1], Andreza Pain Marcelino[1], maria.pimentel[1]

[1]Instituto Nacional de Infectologia Evandro Chagas - INI - FIOCRUZ RJ; [2]Instituto René Rachou - FIOCRUZ Minas

Elizabeth Cristina Araujo Ferreira Faulhaber
FIOCRUZ

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Protocol Citation: Elizabeth Cristina Araujo Ferreira Faulhaber, Daniel Moreira Avelar, Gabrielle Baldan Stanciola, Luanna da Silva Ventura, Andreza Pain Marcelino, maria.pimentel 2025. Standardization of the technique 'LOOP-MEDIATED ISOTHERMAL AMPLIFICATION' (LAMP) using the target 18S rDNA for cutaneous leishmaniasis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvw4dj9lmk/v2Version created by Elizabeth Cristina Araujo Ferreira Faulhaber

Protocol status: Working
We use this protocol and it's working

Created: August 28, 2025

Last Modified: August 29, 2025

Protocol Integer ID: 225696

Keywords: 18S rDNA, “Loop-mediated isothermal amplification” , LAMP, Molecular Biology , Cutaneous Leishmaniasis, mediated isothermal amplification, leishmania sample, cutaneous leishmaniasis leishmaniasi, cutaneous leishmaniasi, isothermal amplification, several protocols for molecular testing, reactivity for all leishmania sample, molecular testing, genus leishmania, leishmaniasi, amplifying large amount, polymerase enzyme, protozoa, large amounts of dna, performance of the lamp technique, promising alternative for the diagnosis, amplification, lamp technique

Funders Acknowledgements:
National Institute of Infectology Evandro Chagas
Grant ID: INI - FIOCRUZ
René Rachou Institute -FIOCRUZ
Grant ID: FIOCRUZ MG
Coordination for the Improvement of Higher Education Personnel
Grant ID: CAPES

Abstract

Leishmaniasis are infectious diseases, transmitted by vectors, caused by protozoa of the genus *Leishmania*. Several protocols for molecular testing (conventional PCR - cPCR; and real-time PCR - qPCR) have been evaluated for the diagnosis of cutaneous leishmaniasis (CL), but they are limited to reference centers. A breakthrough in the diagnosis of various infectious diseases has been the development of the technique 'loop-mediated isothermal amplification' - LAMP. This method is capable of amplifying large amounts of DNA under isothermal conditions within 30-60 minutes. Our goal was to evaluate the performance of the LAMP technique targeting 18S rDNA using the WarmStart® Colorimetric LAMP 2x Master Mix (WS) kit and the Bst 2.0 DNA Polymerase enzyme with SYBR® Green I (BstSg). The analytical sensitivity (detection limit) with the WS mix was up to 10 fg in agarose gel and 100 fg visually, with consistent results in duplicates; and using BstSg, the sensitivity was up to 10 pg. In the analytical specificity using WS, there was reactivity for all *Leishmania* samples. However, the samples of *Paracoccidioides brasiliensis* (Pb) and *Sporothrix globosa* (Sg) showed bands in one of the duplicates in the gel, suggesting possible non-specific amplification. Using BstSg, visual reactivity was observed only for *L. braziliensis* (Lb). However, in agarose gel, samples of *L. infantum* (Li), *L. guyanensis* (Lg), and one of the duplicates of *L. amazonensis* (La) showed bands suggestive of amplification. The LAMP is a promising alternative for the diagnosis of leishmaniasis.

Image Attribution

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Materials

EQUIPAMENTS

- Thermo Scientific™ NanoDrop™ 2000 spectrophotometer
- Double boiler water Novus N1030T - New Technique
- 100 µL micropipette
- 10 µL micropipette

MATERIALS

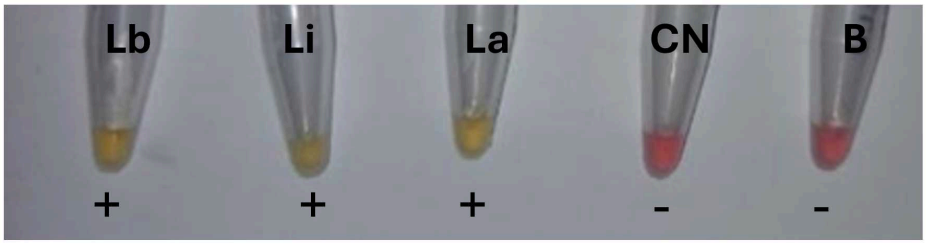
- Sterile microtubes of 1.5 to 2 mL
- Sterile microtubes of 0.5 mL

REAGENTS

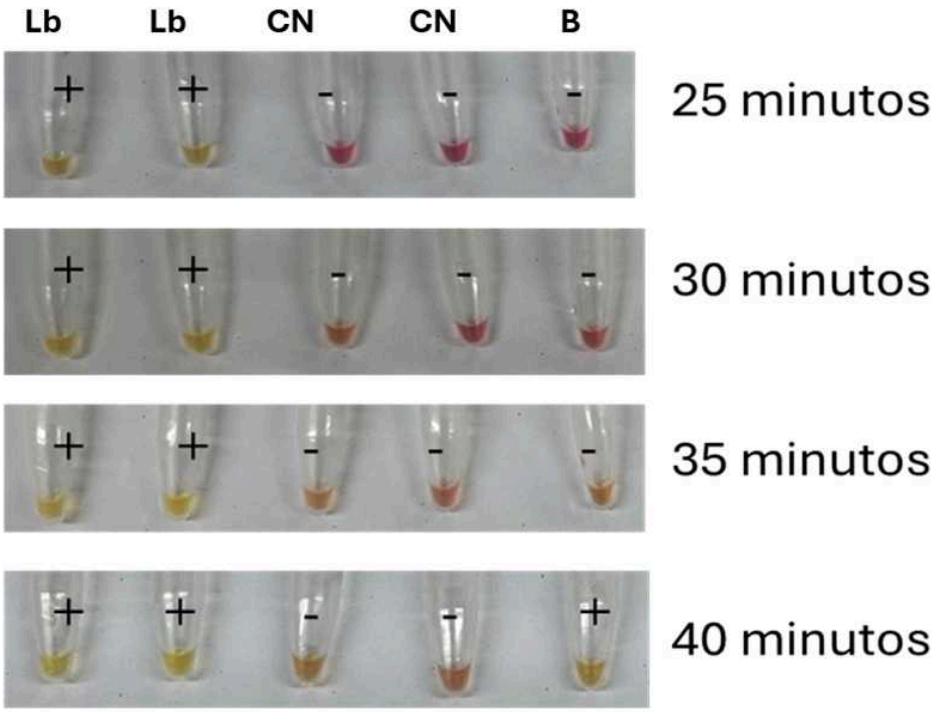
- Phosphorylated Deoxyribonucleotides dNTP's
- Magnesium
- Buffer 1X
- Primers
- Betaine
- *Bacillus stearothermophilus* (BST)
- SYBR® Green I
- kit WarmStart® Colorimetric LAMP 2x Master Mix
- Water
- Deoxyribonucleic acid (DNA)

Standardization assay

1 Standardization assay of LAMP with DNA from reference samples and negative controls using the commercial Warmstart kit (WS) targeting 18S rDNA, with an incubation time of 40 minutes. Tubes containing LAMP reactions with WS for the target 18S rDNA. Lb: *Leishmania* (*Viannia*) *braziliensis*; Li: *leishmania* (*Leishmania*) *infantum*; La: *Leishmania (Leishmania) amazonensis*. CN: negative control with DNA from a negative leishmaniasis patient. B: Negative control with water. (+) Positive reaction; (-) Negative reaction.

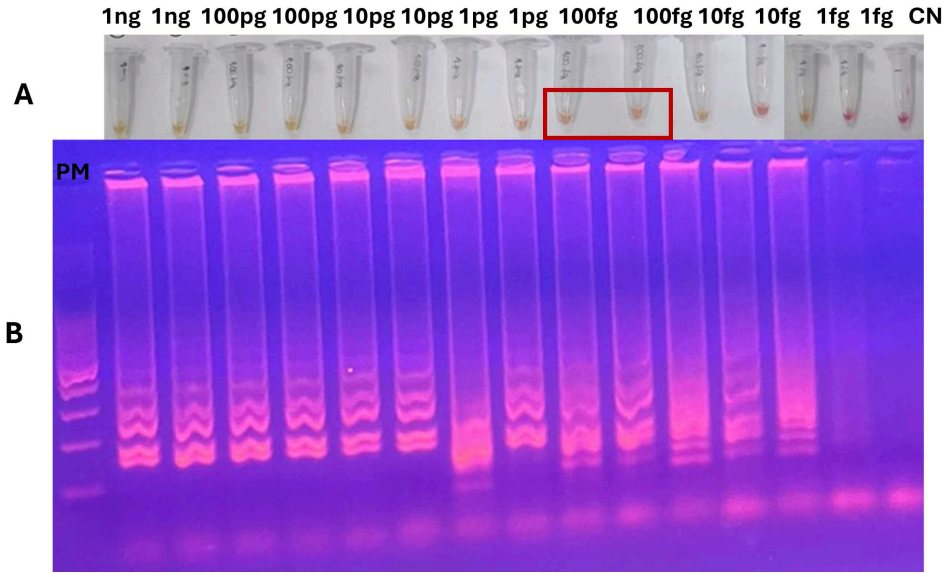


2 Standardization assay of LAMP with DNA from reference samples using WS lot 10265054 targeting 18S rDNA, with incubation times of 25, 30, 35, and 40 minutes. Tubes containing LAMP reactions with WS for the target 18S rDNA. Lb: *Leishmania (Viannia) braziliensis*; CN: negative control with DNA from a patient negative for leishmaniasis. B: Negative control with water. (+) Positive reaction; (-) Negative reaction.



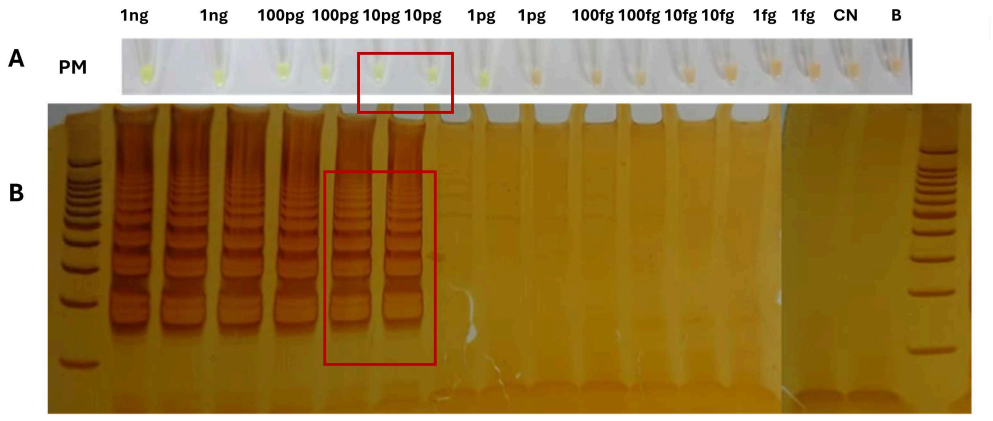
Analytical Sensitivity

3 Analytical sensitivity with WS mix for target 18S rRNA, using a reference sample of *Leishmania* (*Viannia*) *braziliensis* in serial dilutions starting from 1 nanogram. Fig.A.Tubes containing LAMP reactions with WS for the target 18S rDNA at concentrations of 1ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg. CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis. FigB: 2% agarose gel of the products obtained in the tubes illustrated in the reaction of FigA. PM: Molecular weight.



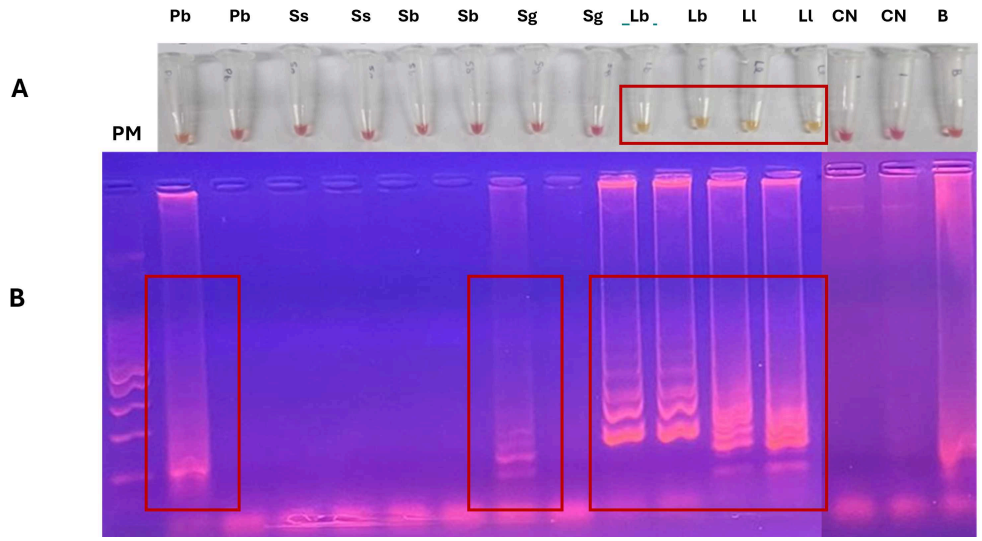
A

3.1 Analytical sensitivity test with *Bst* 2.0 DNA Polymerase com SYBR® Green I (BstSg) for the target 18S rRNA, using a reference sample of *Leishmania* (*Viannia*) *braziliensis* in serial dilutions starting from 1 nanogram. FigA:Tubes containing LAMP reactions with BstSg for the target 18S rDNA at concentrations of 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg. CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis; B-Blank: Negative control with water. FigB: Electrophoresis on polyacrylamide gel of the products obtained from the tubes illustrated in the reaction of FigA. PM: Molecular weight.

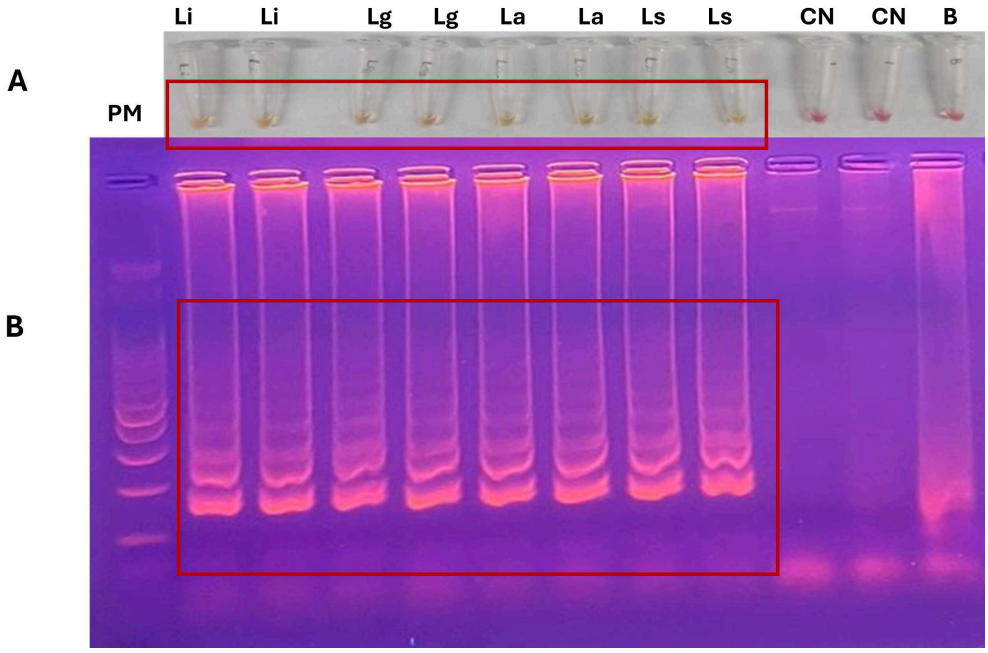


Analytical specificity

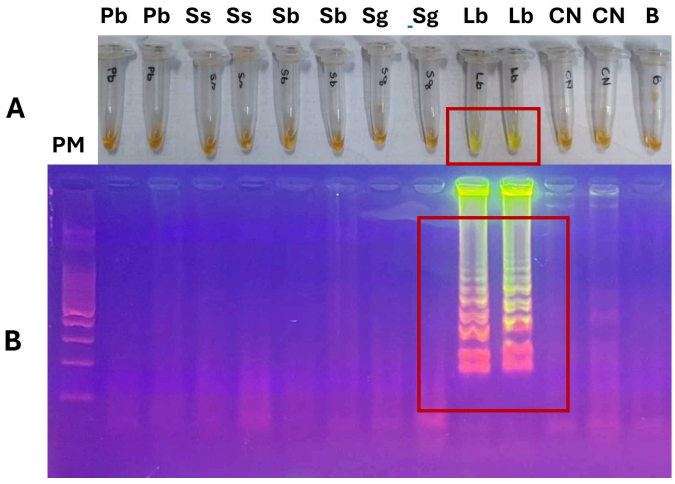
4 Analytical specificity for the target 18Tubes containing LAMP reactions with WS for the target 18S rDNA at a concentration of 5ng/µL. FigA: *Paracoccidioides brasiliensis* (Pb), *Sporothrix schenckii* (Ss), *Sporothrix braziliensis* (Sb), *Sporothrix globosa* (Sg), *Leishmania braziliensis* (Lb), *Leishmania (Viannia) lainsoni* (Ll). CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis. B - Blank: Negative control with water. FigB: 2% agarose gel of the products obtained in the tubes illustrated in the reaction of FigA. PM: Molecular weight.



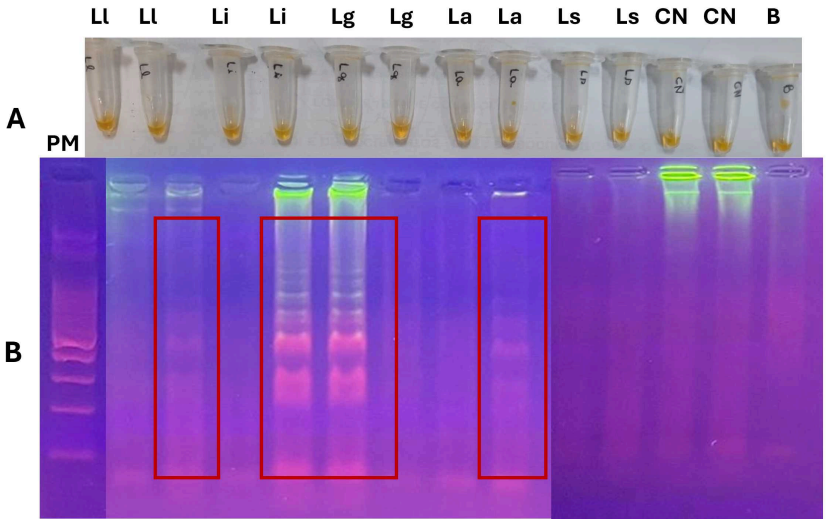
4.1 Analytical specificity for the target 18S rDNA using WS and reference samples of *Leishmania* spp. Tubes containing LAMP reactions with WS for the target 18S rDNA at a concentration of 5ng/µL. FigA.:*Leishmania infantum* (Li), *Leishmania (Viannia) guyanensis* (Lg), *Leishmania amazonensis* (La), and *Leishmania (Viannia) shawi* (Ls). CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis. B - Blank: Negative control with water. FigB.: 2% agarose gel of the products obtained in the tubes illustrated in the reaction of Fig.A. PM: Molecular weight.



4.2 Analytical specificity for the target 18S rRNA using BstSg and reference samples of *Paracoccidioides* spp., *Sporothrix* spp. and *Leishmania* spp. FigA.:Tubes containing LAMP reactions with BstSg for the target 18S rDNA at a concentration of 5ng/µL. *Paracoccidioides brasiliensis* (Pb), *Sporothrix schenckii* (Ss), *Sporothrix brasiliensis* (Sb), *Sporothrix globosa* (Sg), *Leishmania braziliensis* (Lb). CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis. B - White: Negative control with water. Fig.B: 2% agarose gel of the products obtained in the tubes illustrated in the reaction of FigA. PM: Molecular weight.

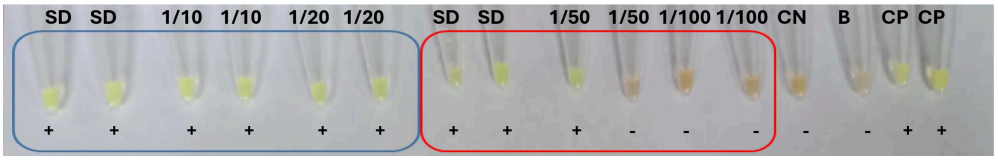


4.3 Analytical specificity for the target 18S rRNA using BstSg, samples of *Leishmania* spp. FigA.: Tubes containing LAMP reactions with BstSg for the target 18S rDNA at a concentration of 5ng/µL. *Leishmania infantum* (Li), *Leishmania guianensis* (Lg), *Leishmania amazonensis* (La), and *Leishmania shawi* (Ls). CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis. B-Blank: Negative control with water. Fig.B: 2% agarose gel of the products obtained from the tubes illustrated in the reaction of Fig.A. PM: Molecular weight.



Performance analysis

5 Performance analysis of BstSg using 18S rDNA in samples of DNA from swabs of skin lesions extracted with a commercial kit and by boiling without dilution and with dilution at 1-10, 1-20 1-50 and 1-100. SD: without dilution; CN: control containing DNA from a patient with a final diagnosis different from leishmaniasis; B-Branco: Negative control with water; CP: Positive control of *L.braziliensis*; (+) Positive reaction; (-) Negative reaction. Marked in blue: DNA samples extracted with the Pure Link® Genomic DNA Mini Kit (30ng/µL) and marked in red: DNA samples extracted by boiling (223,9 ng/µL).



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