

Validation of loop-mediated isothermal amplification for the detection of *Loa loa* infection in *Chrysops* spp in experimental and natural field conditions

Article (Supplemental Material)

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Colorimetric LAMP Protocol for the detection of *L. loa*

1. 25X Primer Mixes:

⁸ Standard Primers	Volume (μ l)	25X concentration	1X concentration
100 μ M FIP	40	40 μ M	1.6 μ M
100 μ M F3	5	5 μ M	0.2 μ M
100 μ M BIP	40	40 μ M	1.6 μ M
100 μ M B3	5	5 μ M	0.2 μ M
H ₂ O	10	----	----
Total Volume	100	----	----

⁸ Loop Primers	Volume (μ l)	25X concentration	1X concentration
100 μ M LF	10	10 μ M	0.4 μ M
100 μ M LB	10	10 μ M	0.4 μ M
H ₂ O	80	----	----
Total Volume	100	----	----

2. Colorimetric LAMP reactions:

Components	Volume (μ l)
2X Warmstart colorimetric Master mix	12.5
25X Standard Primer mix	1
25X Loop primer mix	1
ⁱ Substrate DNA	2
H ₂ O	8.5
Total Volume	25

- a. 100 μ M primer stocks are prepared in H₂O to minimize carry over of Tris.
- b. LAMP reactions are incubated in a GeneAmp®, PCR System 9700 Applied Biosystems @ 61°C for 40 min as described in the Materials and Methods.
- c. Substrate DNA can be dissolved in either elution buffer or H₂O.