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)

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

1. 25X Primer Mixes:

[g]Standard Primers	Volume (µl)	25X concentration	1X concenration
100 µM FIP	40	40 µM	1.6 µM
100 µM F3	5	5 µM	0.2 µM
100 µM BIP	40	40 µM	1.6 µM
100 µM B3	5	5 µM	0.2 µM
H_2O	10	----	----
Total Volume	100	----	----

[g]Loop Primers	Volume (µl)	25X concentration	1X concentration
100 µM LF	10	10 µM	0.4 µM
100 µM LB	10	10 µM	0.4 µM
H_2O	80	----	----
Total Volume	100	----	----

2. Colorimetric LAMP reactions:

Components	Volume (µl)
2X Warmstart colorimetric Master mix	12.5
25X Standard Primer mix	1
25X Loop primer mix	1
[i]Substrate DNA	2
H_2O	8.5
Total Volume	25

a. 100 µM primer stocks are prepared in H_2O 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 H_2O.