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

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## 1. 25X Primer Mixes:

<sup>g</sup> Standard Primers	Volume (µl)	25X concentration	1X concenration
100 μM FIP	40	40 μM	1.6 μΜ
100 μM F3	5	5 μΜ	0.2 μΜ
100 μM BIP	40	40 μM	1.6 μΜ
100 μM B3	5	5 μΜ	0.2 μΜ
H <sub>2</sub> O	10		
Total Volume	100		

<sup>g</sup> Loop Primers	Volume (µl)	25X concentration	1X concentration
100 μM LF	10	10 μM	0.4 μΜ
100 μM LB	10	10 μM	0.4 μΜ
H₂O	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
<sup>i</sup> Substrate DNA	2
H <sub>2</sub> O	8.5
Total Volume	25

a. 100  $\mu M$  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  $H_2O$ .