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Protocol 002: Protocol for *M. Perstans* 419 Colorimetric LAMP

Version Number	Written by	Reviewed by	Approved by
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Principle: The Loop-mediated isothermal amplification (LAMP) assay is a nucleic acid amplification method which amplifies DNA with high specificity and efficiency under isothermal conditions. This technique relies on auto-cycling strand displacement DNA synthesis which is carried out at 60-66 °C for 40-60 minutes in the presence of a DNA polymerase (with high strand displacement activity), dNTPs, specific primers and the target DNA template. It employs a set of four to six specially constructed primers (two inner and two outer primers, and loop primers) that recognize six to eight distinct sequences on the target DNA.

Requirements

Equipment	Consumables	Reagents
<ul style="list-style-type: none"> • Refrigerator (4 °C) • Freezer (-20 °C) • Micro pipettes • Incubator instrument • Vortex mixer • Micro centrifuge 	<ul style="list-style-type: none"> • Tissue paper • Disposable gloves • 1.5 ml and 2 ml micro centrifuge tubes • 0.2 ml 8 strip PCR tubes • Sterile, RNase/DNase free 10 µl, 200 µl, and 1000µl filter tips. 	<ul style="list-style-type: none"> • 2x WarmStart colorimetric master mix (NEB 1804) • Primers • 10X GuHCl • Molecular biology grade water

Storage of reagents

All reagents should be stored at –20 °C

Test Procedure

Before you begin:

- Prepare template worksheet.
- Clean the water bath or heat block or any instrument that can maintain heat at isothermal temperature.
- In PCR Clean room hood, gather reagents for the LAMP and allow them to thaw completely before use. Mix the contents well before use.

Reaction setup

1. *M. perstans* LAMP primers:

Primer	Sequence (5' to 3')
Mpe_FIP (F1c + F2)	TGTGAGCACATTTTCAGTAAGT- GATGAAATCCACTAAATTCWC
Mpe_BIP (B1 + B2c)	GGATTCTTTCTAAAAGTTGAG- GATCGATTTTCGTTAAAAACAGY
Mpe_F3	ACAGTTGATTATTTGAAGGTGCTR
Mpe_B3	AYAATGATTATTTYTAAAGAATC
Mpe_LF	AGACTTGATTACTGTTTGG
Mpe_LB	ACAATTTGGTAATCGCTTAAACTG

- Sequences: *Poole et al. Sci Rep. 2019 Jul 16;9(1):10275. doi: 10.1038/Liz Li designed the loop primers and modified the protocol to improve the speed and sensitivity of the assay.*
- Reaction speed can be improved using HPLC purified primer, but desalted primer showed better sensitivity
- **Dissolve primers to 100 µM in nuclease free water**

N.B Primer stocks are prepared in nuclease-free water and can be stored at –20 °C for up to 2 years

1. Prepare 10X Mpe LAMP Primer Mix:

Primer	10X Concentration (μM)	μl of 100 μM primer stock
100 μM FIP	16	16
100 μM BIP	16	16
100 μM F3	2	2
100 μM B3	2	2
100 μM LF	4	4
100 μM LB	4	4
H ₂ O	-----	56
Total volume (μl)	-----	100

2. Thaw LAMP Master Mix, LAMP Primer Mix and GuHCl
3. Mix thoroughly by vortexing or inversion, ensure that any precipitation in the M1804 mix is resuspended before use.
4. Keep thawed reagents on ice.
5. Calculate the number of reactions required per assay including NTC, PC and EC Assemble LAMP reactions using the following recipe below:

	Plus DNA	No DNA (non-template control)
WarmStart Colorimetric LAMP 2X Master Mix - M1804	10 μl	10 μl
10X Primer Mix	2.0 μl	2.0 μl
10X GuHCl	2.0 μl	2.0 μl
DNA	2 μl	-----
H ₂ O	4.0 μl	6.0 μl

6. Verify reactions are pink; if yellow, repeat with lower sample volume or adjust sample pH to ~8

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7. Record the pre-amplification color of the reaction mix by scanning or taking a photo of the reaction tubes/plate
8. Place reactions at **63 °C**, incubate for 40 minutes.
9. Allow reactions to cool from 66 °C to room temperature or place tubes on ice for improved color contrast before inspecting or recording the color change of reaction tubes
10. Record the post-amplification color of the reaction vessel: yellow color for positive samples and pink color for negative samples.
- 11. Discard completed reactions **without opening reaction vessels.****

Definitions

- NTC- Non Template Control
- PC- DNA from known positive clinical specimen.
- EC - Extracted-water negative control (DNA extraction performed on distilled water).