Colorimetric RT-LAMP Research group SARS-COV-2 detection protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bk59ky96

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vortex swab sample

1 Vortex swab sample in Viral transport medium

2 Take 200**µl** and transfer to 1,5ml microtube

3 Add 500ul of ethanol reconstituted lysis buffer incubate 10 minutes. Vortex or shake after

4 Add solubilization buffer, and wait 2 minutes, shake or vortex after

5 Add 200**µl** 98% Ethanol shake and use a an intense and brief centrifuge. 5-10 sec to 30 000g. This will avoid clogging in the filter cartrige afterwards.

6 Transfer the supernatant to the Kurabo catrige.

7 Wash with wash buffer and filter 3 times with Kurabo-Mini480.

8 add 50-100 (better 50)**µl of** elution buffer or pcr grade water.

9 filter and you have extracted the viral RNA in the elution tubes. You may store it in -80C or use it directly in the isothermic amplification

10 Isothermal amplification: Make a mix of 5**µl** of eluate sample and 5**µl** of pcr grade H2O and 2,5 **µl** of 10x primer mix in a 1,5ml transparent microtube. Standart primer concentrations of 1·6 µM FIP/BIP, 0·2 µM F3/B3, 0·4 µM FL/BL work just fine.

11 add 12.5 **µl** of colorimetric RT-LAMP master mix. The NEB one or your own. Mix well with the pipette tip.

12 add 50**µl** of mineral oil to seal the reaction. This is essencial. Unsealed reactions are very difficult to detect.

13 Make a photo with your mobile phone. to register base colour, should be red-pink.

14 incubate 30 minutes at 63Cº. Take a photo afterwords. You dshold see the positive reactions as yellow. You can register it measuring the green channel in the RG colourspace (a normalized RGB colourspace). Higher than 83 is positive, loyer or equal is negative. To do this take the median values of the RGB measure with ImageJ, divide R,G,B to R+G+B. That is RG colorspace. You only need the green channel.

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