- 1. In a 1.7 mL microfuge tube with a 2x3 mm piece of Whatman No. 1 paper glued with Kwik-Sil™ to the side, 1 mm from the bottom, mix in:
 - 100 μL saliva
 - 50 μL RNAlaterTM (R0901, Sigma) or 'Homebrew' (see below)
 - 25 μL Proteinase K (10 mg/mL; PB0451, BioBasic)
- 2. Pipette up and down ~20x to mix well
- 3. Incubate at ~26°C for 5 min (but not below 22 °C)
- 4. Incubate at 95°C for 5 min
- 5. Remove liquid by decanting or using a pipette
- 6. Add 1 mL Wash Buffer and invert vigorously 20 times
- 7. Incubate at room temperature for 1 min
- 8. Remove liquid by decanting or using a pipette
- 9. Add 1 mL Wash Buffer, incubate at room temperature for 1 min, then remove liquid (this is a brief rinse) leaving as little Wash Buffer as possible
- 10. Add 50 μ L of LAMP rxn mix using ZI-1 primers. (If a control reaction for RNA extraction from saliva is desired, use RAB7A primers instead)
- 11. Take photo of tube(s) as baseline
- 12. Incubate 65°C for 20 min
- 13. Cool to room temperature (or to 4°C to maximize color difference between negative and positive reactions)
- 14. Examine tubes visually for pink (negative) or yellow (positive) color change

Wash Buffer

1 mM Tris-Cl pH 8.0	T4661-100G (Sigma)
0.1 mM EDTA pH 8.0	E9884-100G (Sigma)
0.1 % Tween-20	P9416-50ML (Sigma)

LAMP mix (per reaction)

25 µL 2x WarmStart® Colorimetric LAMP mix (NEB, M1800L)

5 μL 400 mM guanidine hydrochloride (G3272, Sigma, made fresh from powder)

 $5 \mu L$ ZI-1 10x primers (16 μ M FIP/BIP, 2 μ M F3/B3, 4 μ M LF/LB)

15 uL water

Homebrew RNAlater:

3.54 M ammonium sulfate	A4418-500G (Sigma)
16.6 mM sodium citrate	C8532-500G (Sigma)
13.3 mM EDTA	E9884-100G (Sigma)

Primers

ZI-1-F3	GGATACAACTAGCTACAGAGAA
ZI-1-B3	CCACAAGTTACTTGTACCATAC
ZI-1-FIP	TTGGTAAAGAACATCAGAACCTGAGGCTGCTTGTTGTCATCTC
ZI-1-BIP	CCACCACAACCTCTATCACCTAACCCTCAACTTTACCAGAT
ZI-1-LF	AAGTCATTGAGAGCCTTTGC
ZI-1-LB	GTGGTTTTAGAAAAATGGCATTCCC
RAB7A-F3	ACAGGCCTGGTGCTACAG
RAB7A-B3	CTGCAGCTTTCTGCCGAG

RAB7A-FIP CAATCGTCTGGAACGCCTGCTCCCTACTTTGAGACCAGTGC RAB7A-BIP AAGCAGGAAACGGAGGTGGAGGCCCGGTCATTCTTGTCC

RAB7A-LF ACGTTGATGGCCTCCTTG
RAB7A-LB TGTACAACGAATTTCCTGAACC

Notes:

- For gluing Whatman No. 1 paper, use as little Kwik-Sil™ as possible to maximize surface area exposed to saliva mixture. Ideally, allow Kwik-Sil to cure overnight before using.
- We found item https://cardholepunch.com/products/custom-loyalty-card-hole-punch?variant=7789589790786 (custom shape ID: ND15) worked very well to rapidly punch out Whatman No. 1 pieces, with the tapered end exposed to Kwik-Sil and facing the top of the tube.
- The volume of saliva can be larger than 100 μ L as long as the other components are scaled accordingly.
- We have found that incubation of the saliva mixture with Whatman paper can occur between 26°C and 37°C. Our testing was consistent at 26°C. Lower incubation temperatures (e.g. <22°C) may lead to unreliable results, presumably because the activity of Proteinase K is too low.
- The washing steps are important to remove residual RNAlater soaked in the Whatman paper which can affect the LAMP reaction.
- Use of freshly prepared guanidine hydrochloride solution in the LAMP reaction increases the sensitivity of the colorimetric assay.
- For WHotLAMP assays in 1.7 mL microfuge tubes, the 20 min incubation time at 65°C is important. Extended incubation times (e.g. >25 min at 65°C) can lead to non-specific colorimetric change in the negative control. For saliva samples with high SARS-CoV-2 titers, a colorimetric change can be detected in as little as 10 min at 65°C.
- Cooling tubes after the 65°C incubation step to ~4°C helps to bring out the color contrast between positive and negative colorimetric LAMP results.