

One Hour Covid Test Protocol

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Sample collection, viral inactivation and nucleic acid stabilization

Collect saliva samples using the ORAcollect-RNA OR-100 oral swab kit (DNA Genotek) according to the manufacturer's instructions. Samples can be safely stored and transported at room temperature.

RNA processing and concentration

Transfer 100µl from each collection tube to a 0.2 ml tube. From the prepIT-Q2A (DNA Genotek), add 10 µl reagent AG followed by 20 µl reagent ST. Mix samples via inversion or pipetting up and down. Incubate at room temperature for 15 minutes to allow for a phase separation. Carefully collect 10 µl of the top phase and diluted 10X with molecular biology grade water.

Colorimetric RT-LAMP

Use 1 µL diluted sample as the substrate for the RT-LAMP colorimetric assays.

Two RT-LAMP assays are run for each sample. One assay is for detection of the SARS-CoV-2 E and N genes. The other assay is for detection of human beta actin RNA and serves as a positive control for the presence of (human) nucleic acid.

Briefly, 20 µl reactions were prepared containing 10 µl of WarmStart® Colorimetric LAMP 2X Master Mix (M1800), 2 µl of 10X primer mix (primers acquired from IDT) consisting of 16 µM each FIP and BIP, 2 µM each F3 and B3, and 8 µM each LF and LB, along with 40 mM guanidine hydrochloride (Sigma, G3572) and molecular grade water.

Incubate RT-LAMP reactions at 68°C for 30 minutes using an Anova Culinary Sous Vide Precision Cooker Nano or a heat block. Analyze results: pink indicates negative and yellow indicates positive.