Supplemental Online Content

Congrave-Wilson Z, Lee Y, Jumarang J, et al. Change in saliva RT-PCR sensitivity over the course of SARS-CoV-2 infection. *JAMA*. doi:10.1001/jama.2021.13967

eMethods

eReferences

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eMethods

Specimen collection and processing

Participants presented to the research drive-thru respiratory testing center of Children's Hospital Los Angeles for all sample collections. Under the observation of study staff, participants self-collected 3 mL saliva by allowing saliva to collect in the mouth and then dribbling or gently spitting into a 50 mL sterile container. Participants were instructed to avoid eating, drinking, smoking, or chewing gum/tobacco within the previous 30 minutes. They were also instructed not to cough or clear their throat while providing the saliva samples to avoid contamination with excessive mucus. No preservative was added.[1] Once saliva was collected from the participant, study staff would obtain the nasopharyngeal swab specimen, following CDC guidelines. After performing the test, the swab was placed in a 3 mL tube containing universal transport media. Samples were sent to the laboratory within 1 hour of collection. Both nasopharyngeal swab and saliva specimens were vortexed before storage in a 1.5 mL microcentrifuge tube at 4°C for next day RNA extraction and RT-PCR analysis. Some saliva samples were stored at –80°C for future testing. We previously validated that frozen sample RT-PCR Ct values were within +/-1 cycle compared with fresh samples.

SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR)

We tested for SARS-CoV-2 using the CDC protocol for RT-PCR, currently under Emergency Use Authorization by the Food and Drug Administration.[2] Briefly, total nucleic acid was extracted from 200 µL nasopharyngeal swab or saliva samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA) and eluted to 50 µL of total nucleic acid. RT-PCR reactions were prepared using the 1-Step Taqpath Master Mix (Thermo Fisher, Carlsbad, CA) and primers and probes that target the SARS-CoV-2 N1 and N2 genes and Ribonuclease-P (RNP, internal control) (IDT, Coralville, IA). RT-PCR was performed on QuantStudio 5 (Applied Biosystem, Carlsbad, CA). A positive result for SARS-CoV-2 was defined as cycle threshold (Ct) value less than 40 for both N1 and N2. An inconclusive result was defined as Ct value less than 40 for either N1 or N2 only. A valid result for SARS-CoV-2 detection was determined by RNP using a cut-off of Ct value<32. RT-PCR was performed on saliva samples for all consecutive time points until the first negative or inconclusive result or no additional sample was available.

eReferences

1. Centers for Disease Control and Prevention. Interim Guidelines for Clinical Specimens for COVID-19 https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. Published 2021. Accessed February 23, 2021.
2. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. https://www.fda.gov/media/134922/download. Published 2020. Accessed February 1, 2021.

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