Supplemental Online Content

Liotti FM, Menchinelli G, Marchetti S, et al. Assessment of SARS-CoV-2 RNA test results among patients who recovered from COVID-19 with prior negative results. *JAMA Intern Med*. Published online November 12, 2020. doi:10.1001/jamainternmed.2020.7570

eMethods.

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

Clinical Samples Nasal/oropharyngeal swab (NOS) samples were collected in a universal transport medium (UTM®; Copan Italia S.p.A., Brescia, Italy) and tested with real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays for qualitative or quantitative SARS-CoV-2 RNA detection (see below), according to previously described protocols [1,2]. Procedures to prevent sample contamination and PCR carryover were in accordance with standard laboratory practices. In addition, serum samples obtained from blood draws were used for serological testing (see below).

Total SARS-CoV-2 RNA Detection and Quantification Total viral RNA was extracted from NOS samples using the Seegene Nimbus automated system, which uses STARMag Universal Cartridge kit for both RNA extraction and PCR assay setup [1]. Then, RT-PCR testing was performed using the Korea Ministry of Food and Drug Safety approved Seegene Allplex 2019-nCoV assay (Arrow Diagnostics, Genova, Italy)—a single-tube assay targeting three viral genes (E, RdRP, and N), as in World Health Organization recommended protocols, and running on a Bio-Rad CFX96 Real-time Detection system [1]. Each RT-PCR assay provided a cycle threshold (C_T)—the number of cycles required for the fluorescent signal to cross the threshold for a positive assay—and the Seegene automated data analysis software (Seegene Viewer) was used to identify positive detections. In particular, a positive result (i.e., a C_T less than 40) for at least one of two viral genes (i.e., RdRP and N) or for the E gene alone indicates, respectively, the certain or presumptive presence of SARS-CoV-2 RNA in the NOS sample.

Total viral RNA quantification was performed using the Quanty COVID-19 assay (Clonit S.r.l, Milan, Italy) [1]. Separate PCR-microplate wells were each filled with 5-µl sample's extracted RNA (i.e., derived from the Nimbus RNA extraction step), positive control, negative control, and standards. The viral load in the NOS sample was calculated by interpolation of the corresponding C_T value with a standard curve, which was previously built with the C_T values obtained following PCR amplification of samples containing 10^1, 10^2, 10^3, 10^4, and 10^5 copies/µl of synthetic viral N1-encoding RNA, respectively [1].

Replicative SARS-CoV-2 RNA Detection To assess the presence of subgenomic viral RNA (i.e., E gene replicative/intermediate RNA), which is intended as a surrogate for virus replication, samples were subjected to an in-house RT-PCR assay, as described elsewhere [2]. Specifically, using the QIAGEN® oneStep RT-PCR Kit (Qiagen, Valencia, CA, USA), 600 nM concentrations of each of two primers (sgE_SARS-CoV2_F 5'-CGATCTCTTGTAGATCTGTTCTC-3'; sgE_SARS-CoV2_R 5'-ATATTGCAGCAGTACGCACACA-3') and 200 nM concentration of probe (sgE_SARS-CoV2_P 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ-3') were used in a 25-µl reaction volume. Thermal cycling consisted of 30 min at 50°C for reverse transcription, followed by 15 min at 95°C and subsequent 45 cycles of 10s at 95°C, 15s at 55°C, and 5s at 72°C.

Serological Testing Serum samples were tested with a commercial assay (Euroimmun; Lübeck, Germany), an enzyme-linked immunosorbent assay (ELISA) that uses the recombinant S1 domain of the SARS-CoV-2 spike (S) protein as antigen, for semi-quantitative detection of anti–SARS-CoV-2 IgG and IgA antibodies. According to the manufacturer's instructions, results were assessed by calculation of a ratio of the extinction of the control or serum sample over the extinction of the calibrator. This ratio was then interpreted as follows: <1.1, negative; ≥1.1, positive.

References

1. Liotti FM, Menchinelli G, Marchetti S, et al. Evaluation of three commercial assays for SARS-CoV-2 molecular detection in upper respiratory tract samples. *Eur J Clin Microbiol Infect Dis*. Published online September 4, 2020. doi: 10.1007/s10096-020-04025-0
2. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-469. doi: 10.1038/s41586-020-2196-x