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Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription–Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19

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ABSTRACT The clinical performances of six molecular diagnostic tests and a rapid antigen test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were clinically evaluated for the diagnosis of coronavirus disease 2019 (COVID-19) in self-collected saliva. Saliva samples from 103 patients with laboratory-confirmed COVID-19 (15 asymptomatic and 88 symptomatic) were collected on the day of hospital admission. SARS-CoV-2 RNA in saliva was detected using a quantitative reverse transcription-PCR (RT-qPCR) laboratory-developed test (LDT), a cobas SARS-CoV-2 high-throughput system, three direct RT-qPCR kits, and reverse transcription–loop-mediated isothermal amplification (RT-LAMP). The viral antigen was detected by a rapid antigen immunochromatographic assay. Of the 103 samples, viral RNA was detected in 50.5 to 81.6% of the specimens by molecular diagnostic tests, and an antigen was detected in 11.7% of the specimens by the rapid antigen test. Viral RNA was detected at significantly higher percentages (65.6 to 93.4%) in specimens collected within 9 days of symptom onset than in specimens collected after at least 10 days of symptoms (22.2 to 66.7%) and in specimens collected from asymptomatic patients (40.0 to 66.7%). Self-collected saliva is an alternative specimen option for diagnosing COVID-19. The RT-qPCR LDT, a cobas SARS-CoV-2 high-throughput system, direct RT-qPCR kits (except for one commercial kit), and RT-LAMP showed sufficient sensitivities in clinical use to be selectively used in clinical settings and facilities. The rapid antigen test alone is not recommended for an initial COVID-19 diagnosis because of its low sensitivity.

KEYWORDS SARS-CoV-2, saliva, RT-qPCR, RT-LAMP, antigen test

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TABLE 1 Summary of results of molecular diagnostic tests and the rapid antigen test for COVID-19 used on self-collected saliva samples

Test and primer set, method, or target	Total no. (%) of samples (95% confidence interval) (n = 103)	Total no. (%) of samples (95% confidence interval) at the indicated time of collection since the onset of symptoms		
		Early phase (≤9 days) (n = 61)	Late phase (>9 days) (n = 27)	No specific time (asymptomatic) (n = 15)
RT-qPCR LDT[a]	84 (81.6) (72.7–88.5)	57 (93.4) (84.1–98.2)	17 (63.0) (42.4–80.6)	10 (66.7) (38.4–88.2)
N-1 set[e]	76 (73.8) (64.2–82.0)	54 (88.5) (77.8–95.2)	14 (51.9) (31.9–71.3)	8 (53.3) (26.6–78.7)
N-2 set[e]	83 (80.6) (71.6–87.7)	57 (93.4) (84.1–98.2)	16 (59.3) (38.8–77.6)	10 (66.7) (38.4–88.2)
cobas SARS-CoV2 test	83 (80.6) (71.6–87.7)	56 (91.8) (81.9–97.3)	18 (66.7) (46.0–83.5)	9 (60.0) (32.3–83.7)
Target 1	76 (73.8) (64.2–82.0)	54 (88.5) (77.8–95.2)	14 (51.9) (31.9–71.3)	8 (53.3) (26.6–78.7)
Target 2	83 (80.6) (64.2–82.0)	56 (91.8) (81.9–97.3)	18 (66.7) (46.0–83.5)	9 (60.0) (32.3–83.7)
Direct RT-qPCR				
Method A[b]	79 (76.7) (67.3–84.5)	53 (86.9) (75.8–94.2)	16 (59.3) (38.8–77.6)	10 (66.7) (38.4–88.2)
Method B[c]	81 (78.6) (69.5–86.1)	55 (90.2) (79.8–96.3)	17 (63.0) (42.4–80.6)	9 (60.0) (32.3–83.7)
N-1 set[f]	80 (77.7) (68.4–85.3)	54 (88.5) (77.8–95.3)	17 (63.0) (42.4–80.6)	9 (60.0) (32.3–83.7)
N-2 set[f]	63 (61.2) (51.1–70.6)	48 (78.7) (66.3–88.1)	8 (29.6) (13.8–50.2)	7 (46.7) (21.3–73.4)
Method C[d]	52 (50.5) (40.5–60.5)	40 (65.6) (52.3–77.3)	6 (22.2) (8.6–42.3)	6 (40.0) (16.3–67.7)
N-1 set[e]	15 (14.6) (8.4–22.9)	9 (14.8) (7.0–26.1)	2 (7.4) (1.0–24.3)	4 (26.7) (7.8–55.1)
N-2 set[e]	51 (49.5) (39.5–59.5)	40 (65,6) (52.3–77.3)	6 (22.2) (8.6–42.3)	5 (33.3) (11.8–61.6)
RT-LAMP	73 (70.9) (61.1–79.4)	52 (85.2) (73.8–93.0)	12 (44.4) (25.5–64.7)	9 (60.0) (32.3–83.7)
Rapid antigen test	12 (11.7) (6.2–19.5)	8 (13.1) (5.8–24.2)	2 (7.4) (1.0–24.3)	2 (13.3) (1.7–40.5)

[a]LDT, laboratory-developed test.
[b]Method A, SARS-CoV-2 direct detection RT-qPCR kit (TaKaRa Bio Inc., Kusatsu, Japan).
[c]Method B, Ampdirect 2019 novel coronavirus detection kit (Shimadzu Corporation, Kyoto, Japan).
[d]Method C, SARS-CoV-2 detection kit (Toyobo, Osaka, Japan).
[e]Primer and probe set recommended by the National Institute of Infectious Diseases (NIID) in Japan.
[f]Primer and probe set recommended by the Centers for Disease Control and Prevention (CDC) in the United States.

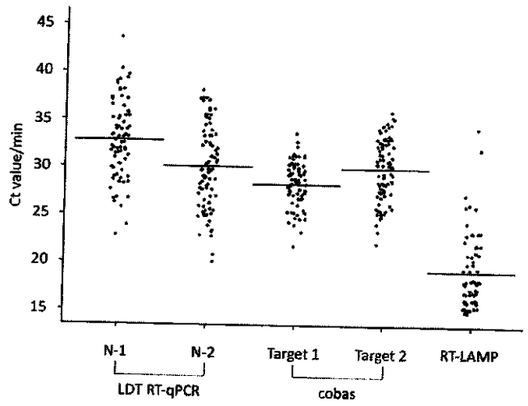

FIG 1 Cycle threshold (C_T) values and detection times for each molecular diagnostic test of saliva specimens. C_T value for each RT-qPCR primer set and detection time by reverse transcription–loop-mediated isothermal amplification (RT-LAMP). Horizontal lines indicate the mean C_T value or detection time.

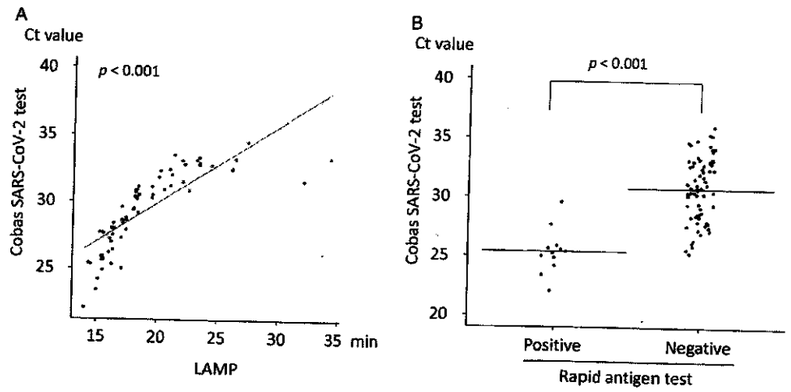

FIG 2 Relation of RT-qPCR, RT-LAMP, and the rapid antigen test (RAT) results for saliva specimens. (A) Relation between the detection time of reverse transcription–loop-mediated isothermal amplification (RT-LAMP) and the C_T value of target 2 (SARS-CoV-2 envelope gene) in the cobas SARS-CoV-2 test. The blue slope line represents the fitted regression curve. The gray shadow indicates the 95% confidence interval around the regression curve. (B) Distribution of the C_T values of target 2 for the cobas SARS-CoV-2 test of saliva with positive and negative results. Horizontal lines indicate the mean C_T value. The P value was calculated using Student's t test.