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SARS-CoV-2 Omicron detection by antigen tests using saliva

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ABSTRACT

The Omicron emerged in November 2021 and became the predominant SARS-CoV-2 variant globally. It spreads more rapidly than ancestral lineages and its rapid detection is critical for the prevention of disease outbreaks. Antigen tests such as immunochromatographic assay (ICA) and chemiluminescent enzyme immunoassay (CLEIA) yield results more quickly than standard polymerase chain reaction (PCR). However, their utility for the detection of the Omicron variant remains unclear. We herein evaluated the performance of ICA and CLEIA in saliva from 51 patients with Omicron and 60 PCR negative individuals. The sensitivity and specificity of CLEIA were 98.0% (95%CI: 89.6–100.0%) and 100.0% (95%CI: 94.0–100.0%), respectively, with fine correlation with cycle threshold (Ct) values. The sensitivity and specificity of ICA were 58.8% (95%CI: 44.2-72.4%) and 100.0% (95%CI: 94.0–100.0%), respectively. The sensitivity of ICA was 100.0% (95%CI: 80.5–100.0%) when PCR Ct was less than 25. The Omicron can be efficiently detected in saliva by CLEIA. ICA also detects high viral load Omicron using saliva.

1. Introduction

Third year into the pandemic, SARS-CoV-2 continues to provide us with challenges by producing new variants. Owing its high transmissibility to shortened incubation time [3] and characterized by many mutations in the spike proteins, Omicron, the most recent variant of concern (VOC), has become the predominant SARS-CoV-2 variant worldwide [1,2]. Notwithstanding the importance of early detection in preventing community outbreaks of COVID-19, the time-consuming reverse transcription quantitative polymerase chain reaction (RT-PCR) remains to be the "gold standard" that may hamper rapid decision making [4–6]. In contrast, rapid antigen tests offer quick, inexpensive, and laboratory-independent point-of-care diagnosis. However, most of these tests were developed prior to the emergence of VOCs and target the nucleocapsid (N) protein [7] which is known to be significantly mutated in Omicron. Omicron is the only major variant with deletions in the N protein and may thereby evade detection by antigen testing.

Self-collected saliva may be as useful a specimen as nasopharyngeal swabs, providing further expeditious screening at lower cost [8–15]. Therefore, we herein evaluated the utility of immunochromatographic assay (ICA) and chemiluminescent enzyme immunoassay (CLEIA) in comparison with RT-PCR.

2. Materials and methods

2.1. Design

This is a prospective study that compares the utility of CLEIA and ICA test against RT-PCR. All assays and screening of SARS-CoV-2 vari-

Abbreviations: ALP, alkaline phosphatase; CLEIA, chemiluminescent enzyme immunoassay; Ct, cycle threshold; ICA, immunochromatographic assay; NPS, nasopharyngeal swab; PCR, polymerase chain reaction; RT-PCR, Real-time reverse transcription–quantitative PCR; VOC, variant of concern.

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ants were conducted simultaneously. Saliva samples were collected from consecutive hospitalized patients with clinically confirmed COVID-19 by the presence of symptoms and PCR tests, as well as symptomatic and asymptomatic close contacts of COVID-19 patients at Hokkaido University Hospital. This study was approved by the Institutional Ethics Board of Hokkaido University Hospital (#020-0116) and informed consent was obtained from all individuals orally to avoid spreading of the virus. Transfer of all physical material (including paper forms) from the COVID-19 infected zone was strictly restricted in order to prevent fomite transmission. Consequently, all information including consent was obtained orally and transferred to written/digital medical records outside the isolation area. This study was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects.

2.2. Saliva samples

Saliva samples were self-collected using a sterile PP Screw cup 50 (Asiakizai, Tokyo, Japan), as described previously [1]. Samples were processed immediately as below. 200 mL of saliva was added to 600 mL PBS, mixed vigorously, then centrifuged at 20,000 × g for 5 min at 4 ˚C to remove debris. The supernatant was used as a sample for testing.

2.3. RT-PCR

Ampdirect™ 2019-nCoV detection kit (Shimadzu, Kyoto, Japan) or TrexGene$_{TM}$ SARS-CoV-2 detection kit (Toyobo, Fukui, Japan) was used to detect SARS-CoV-2 RNA. With Ampdirect™ 2019-nCoV detection kit, 5 μL of sample and 5 μL of sample treatment reagent were mixed, heated for 5 min at 90 ˚C, and cooled on ice. The treated mixture was mixed with 15 μL of reaction mixture containing polymerase, dNTP, primers, and probes. One-step RT-PCR was performed with LightCycler® 96 system (Roche, Basel, Switzerland). Primers of 2019-nCoV-N1-F (5'-GACCCCAAAATCAGCGAAAT-3'), 2019-nCoV-N1-R (5'-TCTGGTTACTGCCAGTTGAATCTG-3'), 2019-nCoV-N2-F (5'-TTACAAACATTGGCCGCAAA-3'), 2019-nCoV-N2-R (5'-GCGCGACATTCCGAAGAA-3'), and probes of 2019-nCoV-N1-P (5'-ROX-ACCCCGCATTACGTTTGGTGGACC-BHQ2-3') and 2019-nCoV-N2-P (5'-FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3') were described in the U.S. CDC's "2019-Novel Coronavirus Real-time rRT-PCR Panel Priers and Probes".

With TrexGene$_{TM}$ SARS-CoV-2 detection kit, 8 μL of sample and 3 μL of sample treatment reagent were mixed and placed for 5 min at room temperature and then heated for 5 min at 95 ˚C. After cooled to room temperature, 40 μL of reaction reagent containing polymerase, dNTP, primers, and probes was added. One-step RT-PCR was performed with LightCycler® 96 system or QuantStudio 5 real-time PCR system (Thermo Fisher Sceintific, Waltham, USA). Primers of 2019-nCoV-N1-F, 2019-nCoV-N1-R, NIID-2019-nCoV-N-F2 (5'-AAATTTTGGGGACCAGGAAC-3'), and 2019-nCoV-N2-R and probes of 2019-nCoV-N1-P (5'-Cy5-ACCCCGCATTACGTTTGGTGGACC-BHQ2-3') and 2019-nCoV_N2-P (5'-ROX-ACAATTTGCCCCCAGCGCTTCAG-BHQ2-3') were described in the U.S. CDC's "2019-Novel Coronavirus Real-time rRT-PCR Panel Priers and Probes" and the National Institute of Infectious Diseases's "pathogen detection manual 2019-nCoV ver.2.9.1".

2.4. Screening of SARS-CoV-2 variants using Sanger sequencing method

RNA was extracted from the saliva samples using Isogen-LS (Nippon Gene, Tokyo, Japan). Briefly, 100 μl of saliva sample from COVID-19 patients were homogenized in 300 μl of Isogen-LS and 80 μl of chloroform was added to the sample, then centrifuged. Following isopropanol precipitation of aqueous phase, the final pellet was dissolved in 40 μl of RNase free water, and cDNA was made using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. A high-efficiency and fidelity DNA Polymerase KOD FX Neo PCR enzyme (Toyobo, Osaka, Japan) was used to amplify the 547 or 1221base pair (bp) fragment of the spike gene from cDNA using the primer set shown in Supplementary Table 1. The amplified fragments were purified using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), then sequenced with Big Dye Terminator kit v3.1(Applied Biosystems, Foster City, CA, USA) with primer G_1R. The resulting sequence was analyzed for genotypes.

2.5. CLEIA

Lumipulse SARS-CoV-2 Ag kit® (Fujirebio Tokyo, Japan), a sandwich CLEIA using SARS-CoV-2 N-Ag monoclonal antibodies on LUMIPULSE G600 (Fujirebio), was performed as described [2]. Antigen levels of > 0.67 pg/mL were defined as positive according to the package insert. 5000 pg/mL was an upper limit of detection. The sample was measured two times and the average value was employed as the antigen

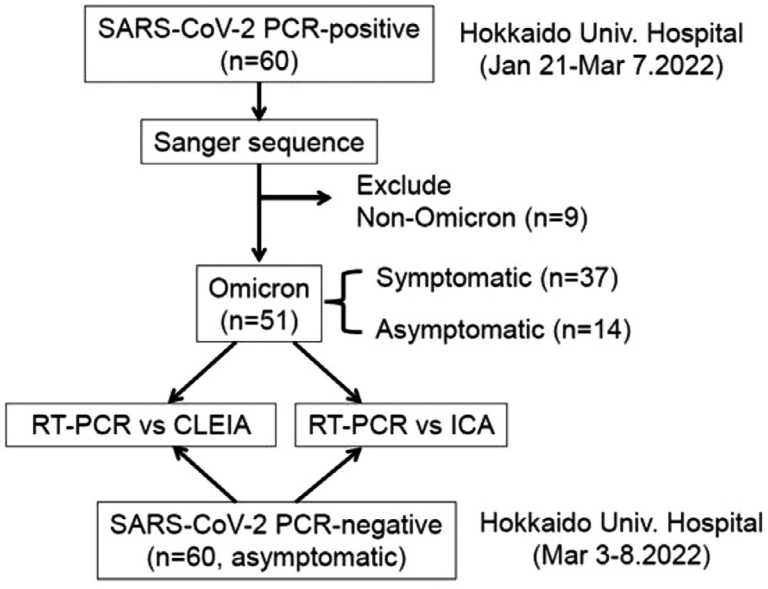

Fig. 1. Flow diagram of participants.

concentration. If one or both detection limits was exceeded, the antigen concentration was defined as exceeding the upper limit of detection.

2.6. ICA

SARS-CoV-2 immunochromatography rapid antigen test (Fujirebio) is an immunochromatography assay based on sandwich enzyme immunoassay methods and makes use of monoclonal antibodies that recognize SARS-CoV-2 N antigen (N-Ag). 100 μL of saliva specimen was treated with 50 μL of concentrated treatment solution (Fujirebio), and 20 μL of the treated specimen was applied onto the cassette and pushed the convex button immediately. SARS-CoV-2 N-Ag in a specimen was captured as an immune-complex with alkaline phosphatase (ALP)-conjugated antibodies by other antibodies immobilized at the test-line position on the membrane. The free ALP-conjugated monoclonal antibodies were captured with the anti-ALP monoclonal antibody immobilized at the reference-line position. These captured ALP-conjugates developed blue lines due to the immersion with the chromogenic substrate in the container in the cassette. Line judgment is carried out 20 min after the convex button was pushed to release the development solution, and judged positive when both the reference and judgment lines were visually confirmed, negative when only the reference line was confirmed; otherwise, no judgment was made.

2.7. Statistical analysis

In this study, we defined RT-PCR diagnosis as the gold standard test. Sensitivity and specificity were calculated for CLEIA and ICA test with 95% Clopper-Pearson exact confidence interval. The correlation between cycle threshold (Ct) value and the CLEIA antigen concentration is shown by a scatter plot and Kendall's coefficient of concordance *W* for RT-PCR positive specimens. A violin plot of CLEIA antigen concentration was also shown for RT-PCR negative specimens. Ct value was plotted and summarized using median and interquartile range stratified by ICA diagnosis. Sensitivity of ICA was also estimated by defining the Ct value of 25 to 34 as conditional RT-PCR positivity to reflect the viral load. The sample size was set at 60 for positive and negative samples, respectively, from feasibility. All statistical analyses were conducted by R 4.1.1 (R Core Team, Vienna, Austria).

Table 1
Characteristics of individuals with Omicron variant confirmed by Sanger sequencing.

Median age (range)		41 (15-77)
Gender	male	24 (47.1%)
	female	27 (52.9%)
Symptoms	yes	37 (72.5%)
	no	14 (27.5%)
Severity (n=37)	mild	34 (91.9%)
	moderate	3 (8.1%)
	Severe	0
Days after symptoms onset (range)		3(1-22)

3. Results

Sixty RT-PCR positive and sixty RT-PCR negative saliva samples were collected at our institution between Jan 21 to Mar 7 and Mar 3 to Mar 8, 2022, respectively (Fig. 1). Among the 60 RT-PCR positive samples, SARS-CoV-2 was identified as Omicron by Sanger sequencing in 51 samples. The remaining 9 samples could not be sequenced most likely due to the small amount of RNA recovery and excluded from the analysis. Of the 51 individuals with confirmed Omicron, 27 (52.9%) were female, with a median age of 41 (range: 15 to 77 years) and thirty-seven (72.5%) were symptomatic. In symptomatic individuals, the median time of sampling was 2 days (range, 1-22 days) after symptom onset (Table 1).

The sensitivity and specificity of CLEIA were 98.0% (95%CI: 89.6–100.0%) and 100.0% (95%CI: 94.0–100.0%), respectively. Kendall's coefficient of concordance of antigen concentrations with CLEIA against Ct values of RT-PCR was 0.98, indicating high correlation between CLEIA and RT-PCR (Fig. 2). The median antigen concentration of CLEIA in RT-PCR negative specimen was 0.10 pg/mL (range: 0.02–0.27 pg/mL).

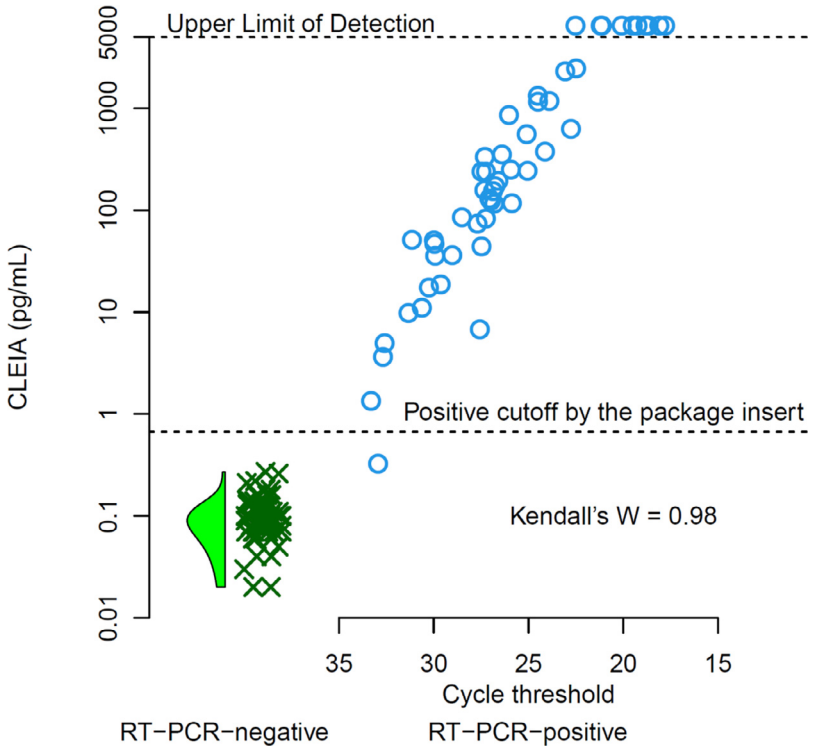

Fig. 2. Comparison of viral load between RT-PCR and CLEIA in saliva specimens. Scatter plot using blue circles between cycle threshold value by RT-PCR and antigen concentration by CLEIA in RT-PCR-positive saliva. Kendall's coefficient of concordance W for RT-PCR positive specimens as nonparametric intraclass correlation coefficient. Scatter plot using green crossed and violin plot of CLEIA antigen concentration in RT-PCR-negative saliva.

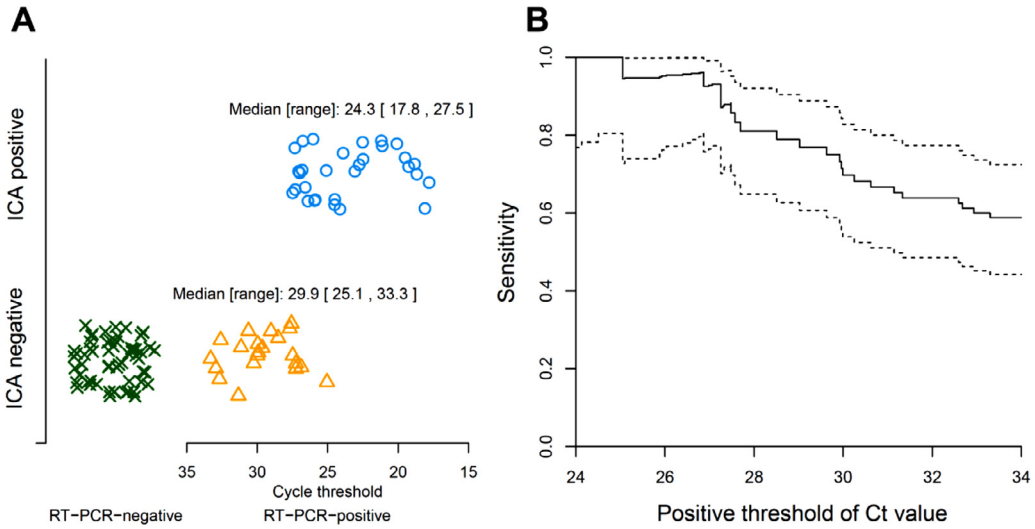

Fig. 3. Comparison of RT-PCR and ICA in saliva specimens, (A) ICA positivity/negativity and Ct values of RT-PCR. Scatter plot, median and range of cycle threshold value by RT-PCR according to diagnosis of ICA. Blue circles and orange triangles represent ICA positive and negative samples, respectively. Green crosses represent RT-PCR negative saliva specimens. (B) Sensitivity when changing the positive cutoff of Ct value by RT-PCR. Sensitivity of ICA against the positive cutoff Ct value of RT-PCR plotted. Solid line and dashed lines indicate point estimates and its 95% confidence intervals, respectively.

The sensitivity and specificity of ICA were 58.8% (95% CI: 44.2–72.4%) and 100.0% (95%CI: 94.0–100.0%), respectively. The median (range) of Ct value by RT-PCR was 24.3 (17.8–27.5) and 29.9 (25.1–33.3) in ICA positive and negative samples, respectively, (Fig. 3A). Thus, ICA could detect SARS-CoV-2 antigens in samples with higher viral load. Sensitivity was 69.8% (95% CI: 53.9–82.8%) for Ct threshold below 30, 92.9% (95% CI: 76.5–99.1%) for Ct threshold below 27, and 100.0% (95% CI: 80.5–100.0%) for Ct threshold below 25 (Fig. 3B).

4. Discussion

We recently demonstrated that CLEIA is reliable alternative to RT-PCR with high concordance between the results of the two tests in 2,056 persons [2]. However, the study was conducted prior to the emergence of Omicron and the effectiveness of CLEIA had been in question in the recent dominance of the Omicron variant.

In this study, we have demonstrated a 100% overall agreement between CLEIA and RT-PCR with 100% sensitivity and specificity. These results are better than those from previous studies performed prior to the prevalence of Omicron. Brandal et al. showed that overall agreement was 95.5% with sensitivity and specificity of 92.3% and 100%, respectively [3].Similarly, we reported an overall agreement of 98.2% with sensitivity and specificity of 76.4% and 99.2%, respectively [2]. Although the number of samples was relatively small herein, we demonstarte that Omicron was efficiently detected in saliva samples using CLEIA,which is less technically demanding and provides results within 30 min [2,3]. CLEIA using self-collected saliva is presently one of the most effective methods for screening large numbers of persons in a short period of time, and has already been implemented at Japanese airport quarantines, facilitating expeditious processing of international travelers with all tests performed at the points of care.

We previously showed that SARS-CoV-2 detection rate was only 24% by ICA using saliva samples before the emergence of Omicron [4]. In the current study, ICA positive rate was 58.8% in overall saliva samples, but was 100.0% for Ct values below 25. Importantly, there was no false positivity. These results suggest that ICA may detect Omicron more effectively than ancestral viral lineages, at least in saliva. However, the small cohort size at a single center may preclude generalization of our results, which requires confirmation in larger cohort studies. Nonetheless, our results suggest that antigen testing could be more useful against Omicron variants than other SARS-CoV-2 variants.

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Declaration of Competing Interest

CLEIA test reagent (Lumipulse SARS-CoV-2 Ag kit), analyzer (LUMIPULSE G600) and ICA test kit were supplied by Fujirebio. IY reports grants from AMED during the conduct of the study; grants from KAKENHI, and Health, Labour and Welfare Policy Research Grants, research fund by Nihon Medi-Physics, and speaker fees from Chugai Pharmaceutical Co, AstraZeneca plt, and Nippon Shinyaku Co, outside the submitted work. TT reports grant from AMED, during the conduct of the study; personal fees from Merck Sharp & Dohme, Takeda Pharmaceutical, Pfizer Japan, and Bristol Myers Squibb, grants and personal fees from Kyowa Hakko Kirin, grants, personal fees, and non-financial support from Novartis Pharma, grants from Chugai Pharmaceutical, Sanofi, Astellas Pharma, Teijin Pharma, Fuji Pharma, Nippon Shinyaku, the Japan Society for the Promotion of Science (Grants-in-Aid for Scientific Research), the Ministry of Health, Labour and Welfare, Japan(Health, Labour and Welfare Policy Research Grants), and the Center of Innovation Program of the Japan Science and Technology Agency, and non-financial support from Janssen Pharmaceutical, outside the submitted work. The other authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

CRediT authorship contribution statement

Kaoru Murakami: Methodology, Data curation, Writing – review & editing. **Sumio Iwasaki:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing. **Satoshi Oguri:** Methodology, Data curation, Writing – review & editing. **Kumiko Tanaka:** Methodology, Data curation, Writing – review & editing. **Rigel Suzuki:** Methodology, Data curation, Writing – review & editing. **Kasumi Hayasaka:** Methodology, Data curation, Writing – review & editing. **Shinichi Fujisawa:** Conceptualization, Methodology, Validation, Data curation, Writing – review & editing. **Chiaki Watanabe:** Methodology, Data curation, Writing – review & editing. **Satoshi Konno:** Methodology, Writing –

review & editing, Project administration. **Isao Yokota:** Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Takasuke Fukuhara:** Methodology, Writing – review & editing, Project administration. **Masaaki Murakami:** Methodology, Writing – review & editing, Project administration. **Takanori Teshima:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100109.

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