Delta Variant (B.1.617.2) of SARS-CoV-2

Subjects: Virology

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Rapid antigen tests (RATs) for COVID-19 based on lateral flow immunoassays are useful for rapid diagnosis in a variety of settings. Although many kinds of RATs are available, their respective sensitivity has not been compared.

SARS-CoV-2 COVID-19 rapid antigen test

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). The WHO reported that more than 230 million cases of COVID-19, including approximately 4.8 million deaths, have occurred as of 29 September 2021 (https://covid19.who.int/). To reduce the burden by SARS-CoV-2, nonpharmaceutical interventions, vaccination, and patient treatment are required. For mitigation of infectious diseases, early and accurate patient diagnosis is essential.

For COVID-19 diagnosis, reverse transcription-quantitative PCR (RT-qPCR) using upper respiratory swabs or saliva has become the gold standard [1] because it possesses high sensitivity and specificity against the target agent. RT-qPCR is usually not available in local clinics where patients who suspect they have COVID-19 go first. Therefore, the collected specimens are transported to sites with RT-qPCR capability, resulting in delayed test results. To obtain results at local clinics, rapid antigen tests (RATs) for COVID-19 have become popular because RATs require just 15–30 min to give results. RATs are also helpful as screening tests for asymptomatic individuals since model analyses showed that population screening tests should prioritize frequency and turnaround time over sensitivity [2][3]. Therefore, RATs might be useful to reduce COVID-19 clusters and spread if frequent self-testing using RATs was performed before mass gatherings, domestic travel, or dining at restaurants. Although the sensitivity of RATs is lower than that of RT-qPCR [4][5][6][7][8][9][10][11], it is essential to utilize RATs with superior sensitivity for better detection.

2. Comparison of Rapid Antigen Tests (RATs)

We evaluated 27 RATs that were available in Japan in September 2021 (**Table 1**). Of these 27 RATs (#1–17), 17 are approved for clinical diagnosis in Japan, whereas the other 10 RATs (#18–27) are not approved for such purpose in Japan. The 27 RATs are divided into three formats: the test strip format, the pen format, and the well format. In the test strip format, a test strip is soaked in lysis buffer containing the specimen or is dipped in the specimen and then soaked in the lysis buffer; the reaction occurs on the strip. In the pen format, the test strip is dipped into the specimen and the reaction occurs on the strip. This format allows saliva specimens to be loaded by

holding the cartridge directly in the mouth. For the well format, lysis buffer containing the specimen is dropped into the well, and the reaction occurs inside a covered plastic body. The well format can be further subdivided into two groups based on how the result is evaluated; for tests #15, #16, and #17, a specific analyzer is required to evaluate the results, whereas the other well-format RATs are assessed by the human eye. Most RATs can process upper respiratory swabs including nasopharyngeal (NP), pharyngeal (P), oropharyngeal (O), or nasal (N) swabs, whereas saliva is the recommended sample for seven RATs (#19, #22, #23, #24, #25, #26, and #27) (**Table 1**). Tests #18, #20, and #21 can be used for both upper respiratory swabs and saliva. Since it is easy for individuals to collect nasal swabs and saliva, the RATs available for such specimens are suitable for self-testing.

Table 1. Characteristics of the rapid antigen tests for COVID-19 evaluated in this study.

No	Rapid Antigen Test	Manufacturer	Country of Origin	Clinical Use in Japan	Format [a]	Recommended Test Sample [b]
1	ESPLINE SARS-CoV-2	Fujirebio	Japan	Yes	Well	NP or N swab
2	ImmunoAce SARS-CoV-2	TAUNS Laboratories	Japan	Yes	Well	NP or N swab
3	Panbio™ COVID-19 Ag Rapid Test Device	Abbott Diagnostics Medical	USA	Yes	Well	N swab
4	PRORAST SARS-CoV-2 Ag	ADTEC/LSI Medience	Japan	Yes	Well	NP or N swab
5	SARS-CoV-2 Rapid Antigen Test	Roche Diagnostics	Switzerland	Yes	Well	NP or N swab
6	Fuji Dry-Chem IMMUNO AG Handy COVID-19 Ag	Fujifilm	Japan	Yes	Well	NP or N swab
7	ALSONIC COVID-19 Ag	Alfresa Pharma	Japan	Yes	Well	NP or N swab
8	COVID-19 and Influenza A+B Antigen Combo Rapid Test	Nichirei Bioscience/Hangzhou AllTest Biotech	Japan/China	Yes	Well	NP or N swab
9	ImmunoArrow SARS-CoV-2	Toyobo	Japan	Yes	Well	NP or N swab

No	Rapid Antigen Test	Manufacturer	Country of Origin	Clinical Use in Japan	Format [a]	Recommended Test Sample [b]
10	Check MR-COV19	Rohto Pharmaceutical	Japan	Yes	Well	NP or N swab
11	RapidTesta SARS-CoV-2	Sekisui Medical	Japan	Yes	Well	NP or N swab
12	QuickNavi-Flu+COVID19 Ag	Denka	Japan	Yes	Well	NP or N swab
13	QuickNavi - COVID19 Ag	Denka	Japan	Yes	Well	NP or N swab
14	KBM LineCheck nCoV	Kohjin Bio	Japan	Yes	Test strip	NP swab
15	BD Veritor System for Rapid Detection of SARS-CoV-2	Becton Dickinson	USA	Yes	Well + Analyzer	N swab
16	Sofia SARS Antigen FIA	Quidel	USA	Yes	Well + Analyzer	NP or N swab
17	Fuji Dri-chem immuno AG cartridge COVID-19 Ag	Fujifilm/Mizuho Medy	Japan	Yes	Well + Analyzer	NP or N swab
18	COVID-19 NP rapid test kit	Shanghai Cagenbio Science	China	No	Well	Saliva or P or O swab
19	SARS-CoV-2 Antigen Rapid Test	Zhuhai Encode Medical Engineering	China	No	Well	Saliva
20	2019-nCoV Ag rapid detection kit	Guangdong Longsee Biomedical	China	No	Well	Saliva or O or NP swab
21	Novel Coronavirus (SARS-CoV-2) Antigen Rapid Test Kit	Beijing Jinwofu Bioengineering Technology	China	No	Well	Saliva or O or NP swab
22	Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit	Jiaxing Wisetest Bio-tech	China	No	Pen	Saliva

No	Rapid Antigen Test	Manufacturer	Country of Origin	Clinical Use in Japan	Format [a]	Recommended Test Sample [b]
23	Corona Virus (COVID-19) Antigen Rapid Test	Hoyotek Biomedical	China	No	Well	Saliva
24	SARS-CoV-2 Antigen Rapid Test Kit	JOYSBIO (Tianjin) Biotechnology	China	No	Well	Saliva
25	Novel coronavirus (2019-nCoV) antigen testing kit	Nanjing Norman Biological Technology	China	No	Well	Saliva
26	COVID19 antigen rapid test device	Toa Industry	Japan	No	Test strip	Saliva
27	Rabliss SARS-CoV-2 antigen detection kit COVID19 AG	Undisclosed	China	No	Well	Saliva

[a] RATs were divided into three types based on their format: (i) well format, in which the lysed sample is dropped into the well and the reaction occurs inside a covered plastic body; (ii) test strip format, in which a test strip is soaked in lysis buffer containing the specimen or dipped in the specimen and then soaked in the lysis buffer, and the reaction occurs on the strip; or (iii) pen format, in which a test strip is dipped into the specimen and the reaction occurs on the strip. “+ Analyzer” means that these RATs need an analyzer to evaluate the result. [b] NP, nasopharyngeal; N, nasal; P, pharyngeal; O, oropharyngeal.

All of the RATs we tested are immunochromatographic tests, meaning that their sensitivity is dependent on the binding kinetics and epitopes of the monoclonal antibodies used in each RAT, the composition of the lysis buffer, the volume of specimen used for analysis, and the method to visualize the result. We cannot directly compare the performance of monoclonal antibodies because the manufacturers do not disclose the properties or amino acid sequence of monoclonal antibodies; however, most RATs likely use monoclonal antibodies against the nucleoprotein of SARS-CoV-2. Because the amino acid sequences of nucleoprotein are similar among human betacoronaviruses, especially the subgenera sarbecovirus, cross-detection is likely to occur against SARS-CoV or SARS-CoV-2-related viruses such as RaTG13 and bat SARS-like coronaviruses. Most of the RATs claim cross-detection of SARS-CoV, with three exceptions: the manufacturers of tests #11 and #15 state that their tests show no cross-reactivity against SARS-CoV, and test #6 cross-detects a high concentration of human coronavirus HKU1 as well as SARS-CoV. Therefore, RATs that show cross-reactivity against SARS-CoV are not able to differentiate patients infected with SARS-CoV-2 and other sarbecoviruses under conditions where these viruses are co-circulating.

The amount of specimen used for each test varied between the RATs (**Table 2**). The input ratio for three RATs with the pen and test strip formats (#14, #22, and #26) was 100% because of the mechanism. Among the well-format tests, the lowest input ratio was for test #20 at 2%, and the highest was for test #24 at 45.7%. According to the detection limits stated in the manufacturers' product information, the RATs could detect SARS-CoV-2 at 35–800 $TCID_{50}$/mL or target virus protein at 10–25 pg/mL (**Table 2**). The results are assessed 5–30 min after adding the analyte (**Table 2**).

Table 2. Rapid antigen tests for COVID-19.

No.	Rapid Antigen Test	Input Rate (%) [a]	Detection Limit [b]	Time to Result (min) [c]
1	ESPLINE SARS-CoV-2	8.0	25 pg/mL	10–30
2	ImmunoAce SARS-CoV-2	13.3	35.6 $TCID_{50}$/test	15
3	Panbio™ COVID-19 Ag Rapid Test Device	14.3	157.7 $TCID_{50}$/mL	15–20
4	PRORAST SARS-CoV-2 Ag	18.2	42 Pfu/mL	15
5	SARS-CoV-2 Rapid Antigen Test	14.3	490 $TCID_{50}$/mL	15–30
6	Fuji Dry-Chem IMMUNO AG Handy COVID-19 Ag	6.0	110 $TCID_{50}$/mL	10
7	ALSONIC COVID-19 Ag	10.9	800 $TCID_{50}$/mL	5
8	COVID-19 and Influenza A+B Antigen Combo Rapid Test	28.6	100 pg/mL	15
9	ImmunoArrow SARS-CoV-2	22.2	25 pg/mL	15
10	Check MR-COV19	21.9	100 $TCID_{50}$/mL	15
11	RapidTesta SARS-CoV-2	21.8	110 $TCID_{50}$/mL	10
12	QuickNavi-Flu+COVID19 Ag	12.5	53 $TCID_{50}$/mL	10
13	QuickNavi -COVID19 Ag	12.5	53 $TCID_{50}$/mL	10
14	KBM LineCheck nCoV	100	625 $TCID_{50}$/mL	10
15	BD Veritor System for Rapid Detection of SARS-CoV-2	26.7	140 $TCID_{50}$/mL	15
16	Sofia SARS Antigen FIA	34.3	113 $TCID_{50}$/mL	15

No.	Rapid Antigen Test	Input Rate (%) [a]	Detection Limit [b]	Time to Result (min) [c]
17	Fuji Dri-chem immuno AG cartridge COVID-19 Ag	23.1	10 pg/mL	15
18	COVID-19 NP rapid test kit	22.2	N.A. [d]	15
19	SARS-CoV-2 Antigen Rapid Test	8.6	N.A.	20
20	2019-nCoV Ag rapid detection kit	2.0	N.A.	15
21	Novel Coronavirus (SARS-CoV-2) Antigen Rapid Test Kit	11.1	100 $TCID_{50}$/mL	15
22	Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit	100	N.A.	15
23	Corona Virus (COVID-19) Antigen Rapid Test	25	N.A.	15
24	SARS-COV-2 Antigen Rapid Test Kit	45.7	160 $TCID_{50}$/mL	15–20
25	Novel coronavirus (2019-nCoV) antigen testing kit	22.9	121 $TCID_{50}$/mL	15–20
26	COVID19 antigen rapid test device	100	N.A.	15
27	Rabliss SARS-CoV-2 antigen detection kit COVID19 AG	10.9	N.A.	8

3. Sensitivity of RATs for SARS-CoV-2 Delta Variant Detection

To compare the sensitivity of the 27 RATs, a delta variant (lineage B.1.617.2) of SARS-CoV-2 was diluted to the indicated PFU and then examined by RT-qPCR to determine the Cq value of each sample. The Cq values were 17.1, 20.9, 24.5, 27.6, and 31.0 at 75,000, 7500, 750, 75, and 7.5 PFU (**Table 3**). Test #22 detected 75 PFU of delta variant in one out of the two tests but failed to detect 7.5 PFU of virus (**Table 3**). Tests #1, #8, #9, and #17 detected 750 PFU of delta variant in both two tests, whereas tests #7, #20, and #27 detected 750 PFU of delta variant in one out of the two tests. Tests #2, #4, #1, and #14 detected 75,000 PFU of delta variant in both two tests but failed to detect 7500 PFU. The other RATs detected 7500 PFU of delta variant. Taken together with the RT-qPCR data, our findings show that the sensitivity for delta variants of tests #1, #7, #8, #9, #17, #20, #22, and #27 is relatively high but lower than that of RT-qPCR.

Table 3. Sensitivity of rapid antigen tests for the delta variant.

No.	Rapid Antigen Test	Virus Titer Tested (PFU/Test)				
		75,000	7500	750	75	7.5
-	RT-qPCR	17.1 [a]	20.9	24.5	27.6	31.0
1	ESPLINE SARS-CoV-2	+ [b]	+	+	–	n.d.
2	ImmunoAce SARS-CoV-2	+	–	–	n.d.	n.d.
3	Panbio™ COVID-19 Ag Rapid Test Device	+	+	–	n.d.	n.d.
4	PRORAST SARS-CoV-2 Ag	+	–	–	n.d.	n.d.
5	SARS-CoV-2 Rapid Antigen Test	n.d. [c]	+	–	–	n.d.
6	Fuji Dry-Chem IMMUNO AG Handy COVID-19 Ag	n.d.	+	–	–	n.d.
7	ALSONIC COVID-19 Ag	n.d.	+	±	–	n.d.
8	COVID-19 and Influenza A+B Antigen Combo Rapid Test	n.d.	+	+	–	n.d.
9	ImmunoArrow SARS-CoV-2	n.d.	+	+	–	n.d.
10	Check MR-COV19	+	–	–	n.d.	n.d.
11	RapidTesta SARS-CoV-2	+	+	–	n.d.	n.d.
12	QuickNavi-Flu+COVID19 Ag	+	+	–	n.d.	n.d.
13	QuickNavi -COVID19 Ag	+	+	–	–	n.d.
14	KBM LineCheck nCoV	+	–	–	–	n.d.
15	BD Veritor System for Rapid Detection of SARS-CoV-2	+	+	–	–	n.d.
16	Sofia SARS Antigen FIA	+	+	–	–	n.d.
17	Fuji Dri-chem immuno AG cartridge COVID-19 Ag	n.d.	+	+	–	n.d.
18	COVID-19 NP rapid test kit	+	+	–	–	n.d.
19	SARS-CoV-2 Antigen Rapid Test	+	+	–	–	n.d.
20	2019-nCoV Ag rapid detection kit	+	+	±	–	n.d.
21	Novel Coronavirus (SARS-CoV-2) Antigen Rapid Test Kit	+	+	–	–	n.d.
22	Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit	n.d.	+	+	±	–
23	Corona Virus (COVID-19) Antigen Rapid Test	+	±	–	–	n.d.
24	SARS-CoV-2 Antigen Rapid Test Kit	+	+	–	–	n.d.

No.	Rapid Antigen Test	Virus Titer Tested (PFU/Test)				
		75,000	7500	750	75	7.5
25	Novel coronavirus (2019-nCoV) antigen testing kit	+	+	–	–	n.d.
26	COVID19 antigen rapid test device	+	+	–	–	n.d.
27	Rabliss SARS-CoV-2 antigen detection kit COVID19 AG	n.d.	+	±	–	–

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