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Immunoassay and chemiluminescent enzyme immunoassay for COVID-19 diagnosis



Toshiaki Ishii ^{a,b}, Masakazu Sasaki ^{a,b}, Kageto Yamada ^{a,b,*}, Daiki Kato ^a,
Hiroyoshi Osuka ^a, Kotaro Aoki ^b, Toshisuke Morita ^{a,c}, Yoshikazu Ishii ^b, Kazuhiro Tateda ^b

^a Department of Clinical Laboratory, Toho University Omori Medical Center, 6-11-1, Omori-nishi, Ota-ku, Tokyo, 143-8541, Japan

^b Department of Microbiology and Infectious Disease, Toho University School of Medicine, 5-21-16, Omori-nishi, Ota-ku, Tokyo, 143-8540, Japan

^c Department of Laboratory Medicine, Toho University Graduate School of Medicine, 5-21-16, Omori-nishi, Ota-ku, Tokyo, 143-8540, Japan

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ABSTRACT

Introduction: The rapid and accurate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is required to prevent the spread of COVID-19. This study evaluated the utility of two SARS-CoV-2 antigen detection methods.

Methods: We evaluated two types of antigen detection methods using immunoassay (Espline) and quantitative chemiluminescent enzyme immunoassay (Lumipulse). RT-PCR was performed as a standard procedure for COVID-19 diagnosis. Lumipulse and RT-PCR were performed for all 486 nasopharyngeal swabs and 136 saliva samples, and the Espline test was performed for 271 nasopharyngeal swabs and 93 saliva samples.

Results: The sensitivity and specificity of the Espline test were 10/11 and 260/260 (100%), respectively for the nasopharyngeal swabs and 3/9 and 84/84 (100%), respectively for the saliva samples. High sensitivities for both saliva (8/9) and nasopharyngeal swabs (22/24) were observed in the Lumipulse test. The specificities of the Lumipulse test for nasopharyngeal swabs and saliva samples were 460/462 (99.6%) and 123/127 (96.9%), respectively.

Conclusion: The Espline test is not effective for saliva samples but is useful for simple and rapid COVID-19 tests using nasopharyngeal swabs because it does not require special devices. The Lumipulse test is a powerful high-throughput tool for COVID-19 diagnosis because it has high detection performance for nasopharyngeal swabs and saliva samples.

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Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is one of the most severe health concerns facing the world today [1]. A COVID-19 diagnosis is determined principally by detecting the viral genome RNA using a quantitative reverse transcription polymerase chain reaction (RT-PCR) in the patients' nasopharynx and saliva [1]. Although RT-PCR has high sensitivity and specificity, it remains difficult to perform in all clinical laboratories because it requires special devices and reagents. While antibody detection is also a

useful tool for investigating past SARS-CoV-2 infection, this method may not be sufficient for the diagnosis of COVID-19 [2]. Antigen detection methods based on immunoassay and fluorescence immunoassay have been developed for the diagnosis of COVID-19 [3]. However, the sensitivity of immunoassay was low (ca. 30.2–75.5%) and a fluorescence analyzer was required for fluorescence immunoassay [3].

More recently, a rapid antigen detection kit, Espline® SARS-CoV-2 (Espline; Fujirebio Inc., Tokyo, Japan), was developed for the detection of the viral nucleocapsid antigen based on immunoassay [4]. Additionally, a quantitative antigen detection reagent with high sensitivity and specificity, Lumipulse® SARS-CoV-2 (Lumipulse; Fujirebio Inc.) based on a chemiluminescent enzyme immunoassay of nasopharyngeal swabs and saliva samples, was developed [5]. However, the information regarding

Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; RT-PCR, quantitative reverse transcription polymerase chain reaction; COVID-19, coronavirus disease 2019.

* Corresponding author. Department of Clinical Laboratory, Toho University Omori Medical Center, 6-11-1 Omori-nishi, Ota-ku, Tokyo, 143-8541, Japan.

E-mail address: kageto.yamada@med.toho-u.ac.jp (K. Yamada).

Table 1

Results of each antigen detection methods compared with the RT-PCR: a. clinical evaluation of the Espline test; b. clinical evaluation of the Lumipulse test.

(a) Espline test							
Sample type		RT-PCR		Predictive values	Sample type		Predictive values
		Positive	Negative				
Nasopharyngeal swabs					Saliva		
Espline	Positive	10	0	100%	Positive	3	0
	Negative	1	260	99.6%	Negative	6	84
	Total	11	260		Total	9	84
Sensitivities/specificities		90.9%	100%			33.3%	100%
(b) Lumipulse test							
Sample type		RT-PCR		Predictive values	Sample type		Predictive values
		Positive	Negative				
Nasopharyngeal swabs					Saliva		
Lumipulse	Positive	22	1 ^a	95.7%	Positive	8	1
	Indeterminate	0	1		Indeterminate	1	3
	Negative	2	460	99.6%	Negative	0	123
	Total	24	462		Total	9	127
Sensitivities/specificities		91.7%	99.6%			88.9%	96.9%

^a Previous RT-PCR in another sample for the same patient was positive.

these antigen detection tests for SARS-CoV-2 is limited, especially information with respect to the use of saliva samples. Therefore, this study evaluated the utility of two SARS-CoV-2 antigen detection tests, Espline and Lumipulse, using both nasopharyngeal swabs and saliva samples; these tests were compared to the RT-PCR.

Overall, 486 nasopharyngeal swabs, including 24 RT-PCR-positive samples, and 136 saliva samples, including 9 RT-PCR-positive samples, were collected from 33 COVID-19 patients and 564 non-COVID-19 patients at Toho University Omori Medical Center between August and September 2020. All the samples were used unfrozen and fresh. The Lumipulse test and RT-PCR were performed on all the test samples, while the Espline test was performed on randomly selected 271 nasopharyngeal swabs and 93 saliva samples. Since RT-PCR and the Lumipulse test were given priority in this study, the Espline test could not be performed when there was a shortage of samples. Both the Espline and Lumipulse tests were performed according to the manufacturers' recommendations. To compare the Lumipulse and Espline tests, although the saliva samples were not generally suited to immunochromatography, we tested both saliva and nasopharyngeal samples. The saliva samples were diluted 2-fold with a dedicated reagent. The same sample solution was used in both the Espline and Lumipulse tests. The sample solution was centrifuged at 12,000 rpm for 2 min. The centrifuged supernatant was dropped into the Espline test device, and the result was judged visually after 30 min. The Espline test was judged as positive when the positive line was observed with the naked eye. The Lumipulse test was used for quantitative antigen detection on the Lumipulse G1200 system (Fujirebio) for 35 min. The Lumipulse test measurement range was from 0.6 to 5000 pg/mL. A previous report indicated that the correlation between the virus load determined using RT-PCR and antigen amount determined using the Lumipulse test was maintained up to the antigen amount of 10,000 pg/mL [6]; thus, the antigen amount >5000 pg/mL was not diluted in this study. According to the manufacturer's recommendations, the criteria for the Lumipulse test were as follows: positive (nasopharyngeal >10 pg/mL; saliva >4.0 pg/mL), indeterminate (nasopharyngeal, 1.0–10 pg/mL; saliva, 0.67–4.0 pg/mL), and negative (nasopharyngeal <1.0 pg/mL; saliva <0.67 pg/mL). The RT-PCR was performed as previously described using the TaqMan Fast Virus 1-step Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) on the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) [7]. The viral RNA was extracted

from the Lumipulse test suspension using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). A threshold cycle (Ct) value less than 35 was positive, and a Ct value of 35 or more was defined as below the limit of detection (LOD) [7].

Table 1 shows the results of each detection method. The Espline test for nasopharyngeal swabs was highly sensitive (10/11 were positive) and had high specificity (260/260, 100%), in addition to its high concordance with the RT-PCR results. In one false-negative result, the Ct value of the RT-PCR was 33.7 because of a low virus load. Conversely, the Espline test for saliva samples showed a significantly low sensitivity (3/9 were positive), suggesting that the Espline test was not suitable for saliva samples. According to previous reports on immunochromatography using nasopharyngeal swabs, the sensitivities were 30.2%–57.6% for the Respi-strip (CORIS BioConcept, Gembloux, Belgium) and 73.3%–75.5% for the Panbio COVID-19 Rapid Test Device (Abbot, Chicago, USA) [3,8–10]. These previous reports on the diagnosis of COVID-19 using immunochromatography showed lower sensitivities than of our study. The high sensitivity and specificity of the Espline test may be related to the use of centrifugation to process the sample. However, the main purpose of centrifugation is to remove impurities and increase specificity, which may not significantly affect sensitivity. In addition, previous reports have indicated that high sensitivities were obtained during the first week or within nine days of symptom onset and with high viral loads [4,8]. Therefore, the sensitivity of immunochromatography might depend on the timing of sampling. Although we studied only small numbers of positive samples, the Espline test may be useful for the diagnosis of COVID-19 using nasopharyngeal swabs. In addition, since the Espline test does not need special devices, it has a shorter turnaround time and is easy to judge.

The sensitivity and specificity of the Lumipulse test for nasopharyngeal swabs were 22/24 (2 negative) and 460/462 (99.6%, 1 positive and 1 indeterminate), respectively. Although a sample that gave the false-positive result with the Lumipulse test had a 27.3 pg/mL antigen value and was below the LOD for the RT-PCR, SARS-CoV-2 RNA was detected using a RT-PCR when the same patient was tested again four days earlier. This patient was diagnosed with COVID-19 four days earlier with an antigen level of 20.9 and a Ct value of 33.7. Thus, this result was considered a false-negative for the RT-PCR, which may have been due to extraction of the RNA from the antigen test suspension. One indeterminate result showed

Table 2
Detailed testing results of the coronavirus disease 2019 patients. a. nasopharyngeal swab samples; b. saliva samples. Symptomatic means dysosmia, dysgeusia, fever, and pneumonia. Asymptomatic patients were close contact to coronavirus disease 2019 patients.

(a)

Sample type, nasopharyngeal swab				Elapsed days ^a	RT-PCR		Lumipulse		Espline	Symptomatic or asymptomatic
Serial no.	Age	Gender	Underlying disease		Ct value	Judgement	Antigen value (pg/mL)	Judgement		
1	50s	Male	Diabetes	0	17.2	+	>5000	+	+	Symptomatic
2	40s	Male	Unknown	4	22.8	+	>5000	+	N/A	Symptomatic
3	20s	Female	Tonsillitis	2	24.3	+	>5000	+	+	Symptomatic
4	50s	Male	Asthma, hypertension	1	14.6	+	>5000	+	N/A	Symptomatic
5	20s	Male	Unknown	0	19.1	+	>5000	+	N/A	Symptomatic
6	20s	Female	Unknown	0	18.3	+	>5000	+	+	Symptomatic
7	50s	Male	Upper extremity deep vein thrombosis	0	20.3	+	>5000	+	+	Symptomatic
8	40s	Male	Hypertension, hyperuricemia, hypercholesterolemia	2	15.6	+	>5000	+	+	Symptomatic
9	90s	Male	Angina, hypertension, aortic dissection	0	21.0	+	>5000	+	+	Symptomatic
10	50s	Male	Diverticulitis	0	14.5	+	>5000	+	N/A	Symptomatic
11	20s	Male	Unknown	N/A ^b	21.9	+	>5000	+	N/A	Asymptomatic
12	30s	Male	Unknown	N/A	29.6	+	>5000	+	N/A	Asymptomatic
13	20s	Female	Unknown	N/A	23.8	+	>5000	+	N/A	Asymptomatic
14	30s	Male	Unknown	1	20.3	+	>5000	+	+	Symptomatic
15	20s	Female	Unknown	0	16.0	+	>5000	+	N/A	Symptomatic
16	30s	Male	Unknown	6	26.5	+	3238.70	+	N/A	Symptomatic
17	80s	Male	Acute myocardial infarction	1	27.8	+	1858.10	+	+	Symptomatic
18	20s	Female	Unknown	N/A	29.4	+	1144.50	+	N/A	Asymptomatic
19	70s	Female	Hypertension, old cerebral infarction	0	34.7	+	526.60	+	+	Symptomatic
20	60s	Female	Asthma	4	31.1	+	362.40	+	+	Symptomatic
21	20s	Female	Asthma	N/A	33.0	+	109.70	+	N/A	Asymptomatic
22	10s	Male	Diabetes	0	33.7	+	20.90	+	–	Symptomatic
23	<10	Male	Kawasaki Disease	0	33.4	+	<0.60	–	N/A	Symptomatic
24	30s	Male	Unknown	N/A	34.1	+	<0.60	–	N/A	Asymptomatic
25	10s	Male	Diabetes	5	>35.0	–	27.30	+	–	Symptomatic

(b)

Sample type, saliva				Elapsed days	RT-PCR		Lumipulse		Espline	Symptomatic or asymptomatic
Serial no.	Age	Gender	Underlying disease		Ct value	Judgement	Antigen value (pg/mL)	Judgement		
1	20s	Male	Unknown	1	18.3	+	>5000	+	+	Symptomatic
2	70s	Male	Pancreatic cancer	0	17.3	+	4791.02	+	+	Symptomatic
3	50s	Female	Lichen planus	9	18.6	+	2090.03	+	+	Symptomatic
4	20s	Male	Paroxysmal supraventricular tachycardia	0	28.1	+	45.02	+	–	Symptomatic
5	20s	Male	Unknown	0	29.1	+	17.96	+	–	Symptomatic
6	20s	Female	Unknown	6	31.4	+	17.34	+	–	Symptomatic
7	70s	Male	Hypertension, diabetes, chronic kidney disease, lung cancer	14	30.6	+	16.64	+	–	Symptomatic
8	30s	Female	Unknown	0	28.8	+	8.48	+	–	Symptomatic
9	20s	Female	Unknown	2	32.6	+	1.04	Indeterminate	–	Symptomatic

^a Onset-day was defined as day 0.

^b N/A, not available.

a very low antigen level (1.0 pg/mL). Two false-negative results revealed high Ct values in RT-PCR (33.4 and 34.1); thus, it was considered that these samples contained only a low virus load.

By contrast, the Lumipulse test for saliva samples showed a higher sensitivity than the Espline test (8/9, 1 indeterminate). The specificity of the Lumipulse test for saliva samples was 123/127 (96.9%, 1 positive and 3 indeterminate). One false-positive result had a 4.54 pg/mL antigen value, and the RT-PCR showed a result below the LOD. Among the four indeterminate results, one result showed that the Lumipulse test and Ct value of RT-PCR were 1.04 pg/mL and 32.6, respectively. In three of the four indeterminate results, all three RT-PCR results were negative, and the antigen quantification values were as follows: 0.71, 0.86, and 3.39 pg/mL.

Our results indicated that the Lumipulse test had high sensitivity and specificity for both nasopharyngeal swabs and saliva samples. Particularly, one nasopharyngeal swab from a SARS-CoV-2 patient was negative in the RT-PCR and positive in the Lumipulse test. Thus, our results suggested that the Lumipulse test might have a high detection performance similar to the RT-PCR. This is the first report to evaluate the usefulness of the Lumipulse test in diagnosing COVID-19 using saliva. Previous studies of the Lumipulse test using nasopharyngeal swabs indicated that the sensitivities and specificities were 55.2%–91.7% and 97.3%–99.6%, respectively [5,11], and that these differences might be dependent on the timing of the sampling mentioned above. The detailed results of the COVID-19 positive patients are shown in Table 2. Almost all the

samples used in this study were obtained under 7 days after onset (Table 2). No association was found between the presence or absence of underlying diseases and testing results. The distribution of the SARS-CoV-2 antigen value in COVID-19 patients were 4.5 to >5000, and the geometric mean was 808.9 pg/mL. If positive results with low antigen value or indeterminate results are observed, it may be necessary to perform an additional test with using RT-PCR and to assess the patients' symptoms. There have been reports of a case of confusion to a false-positive result from the Lumipulse test [11].

This study had a limitation. A limited number of positive SARS-CoV-2 samples (both nasopharyngeal swabs and saliva samples) were available for this study. Therefore, further evaluations of the Espline and Lumipulse tests might be needed.

In conclusion, although the Espline test could not be used for saliva samples, it is useful for a simple point-of-care testing because it does not require a special device. In contrast, although the Lumipulse test requires a special device and reagents, it is useful in routine diagnosis because the Lumipulse test has a performance similar to the RT-PCR and has a simpler procedure and higher throughput than RT-PCR.

Authorship statement

All authors meet the ICMJE authorship criteria. All authors have made substantial contributions to the conception and design of the study, data acquisition, data analysis and interpretation, drafting or revision of the article for important intellectual content, and final approval of the version to be submitted.

Author contributions

TI helped in writing the original draft; he was also involved in the methodology and formal analysis of this paper. MS, KY, HO, DK, KA, TM, YI, and KT contributed to reviewing and editing and were also involved in the formal analysis and writing of the original draft.

Ethical approval

This study protocol was approved by the Ethics Committee of the Faculty of Medicine, Toho University (No. A20028_A20020_A20014_A19099).

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Declaration of competing interest

None.

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