Short communications

Loop-Mediated Isothermal Amplification for Diagnosing SARS-CoV-2 Infection in Two School Children and a Neonate

Running head: LAMP for SARS-CoV-2 diagnosis in children

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SUMMARY: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is spreading worldwide and is a public health problem. Although real-time reverse-transcription polymerase chain reaction (RT-PCR) is gold standard for diagnosing coronavirus disease (COVID-19) and there are many reports discussing it, reports about loop-mediated isothermal amplification (LAMP) tests for SARS-CoV-2, especially in children, are limited. We report the test results of three children with COVID-19 in a family cluster and assess the results of LAMP tests. The LAMP results of these children showed a sensitivity and specificity of 63.6% and 100%, respectively, that was relative to the RT-PCR results. LAMP tests using nasopharyngeal swab (NPS) and RT-PCR were almost consistent throughout hospitalization in the school children, except in the very early stage of infection. The preliminary results suggest that salivary samples would be less sensitive than NPS for LAMP testing in the late stage of infection, and that LAMP would not provide accurate results in neonates.

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In December 2019, coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, and has spread rapidly worldwide (1). The mortality rate of COVID-19 children is lower than that in older adults (2). Real-time reverse-transcription polymerase chain reaction (RT-PCR) is widely used for detecting SARS-CoV-2. However, RT-PCR is expensive, takes at least two hours to provide the results, and requires trained medical personnel, whereas loop-mediated isothermal amplification (LAMP) amplifies nucleic acid without expensive reagents or instruments, and the results are available in approximately an hour (3-7). Although LAMP is considered useful, there are few reports on using LAMP to diagnose COVID-19 in children. This study aimed to evaluate the use of LAMP for detecting SARS-CoV-2 in children. We performed RT-PCR and LAMP using nasopharyngeal swab (NPS) samples, and immunoglobulin G (IgG) and IgM antibody tests using blood samples, twice a week. We also performed RT-PCR and LAMP using saliva samples whenever necessary; the two school children were asked to spit out 1−2 mL of saliva into sterile containers; the neonate was swabbed inside the mouth, which provided enough saliva for analysis. In all three children, salivary sample collection went smoothly. For RT-PCR, RNA was extracted from clinical samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), and amplified

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and detected using the QuantiTect Probe RT-PCR Kit (Qiagen). However, since RT-PCR was performed at a local health center, we could not obtain information on the viral load. For LAMP, RNA was extracted by placing the NPS in the product solution of the Loopamp Influenza Virus RNA Extraction reagent (Eiken Chemical Co., Ltd., Tokyo, Japan) and stirring it 10 times, and amplified and detected with the Loopamp SARS-CoV-2 Detection Reagent Kit (Eiken Chemical Co., Ltd.). The LAMP detection kit did not perform quantitative RNA testing. Informed consent for the tests was obtained from the guardians. This study was approved by the Sagamihara National Hospital Ethics Committee (2020-011).

A father had contact with a COVID-19 patient and returned home. He was asymptomatic and was in contact with his four children for 4 days (boys aged 8, 6, and 1 year and a neonate girl). Day 1 was defined as the day on which all the children were admitted to our hospital. On day 0, the father tested positive for SARS-CoV-2 through the RT-PCR; thus, the other family members were hospitalized and observed separately from day 1 (Table 1). All children were asymptomatic on admission and did not require treatment. One of the four children did not test positive on any tests, thus, we ruled out COVID-19 in this child. The other three children were diagnosed with COVID-19 using RT-PCR. During this period, their leukocyte and eosinophil counts and C-reactive protein levels remained normal (Table 2).

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Regarding the RT-PCR using NPS, although patient 1 tested negative for SARS-CoV-2 using RT-PCR on day 1, he experienced a loss of taste and could not distinguish roasted green tea from coke; he did not have loss of taste for other dishes. He tested positive on day 6 and was positive for 11 days. Patient 2 tested positive on admission and was positive for 20 days. He experienced a transient cough for a few hours in the morning of day 5. Patient 3 tested positive on day 3 and was positive for a week. Regarding the LAMP using NPS, it was positive from day 10 to day 17 in patient 1, from day 3 to 20 in patient 2, and was positive on day 3 in patient 3 alone. The consistency rate between NPS and saliva results for RT-PCR was 86.7% and 63.6% for LAMP in all three patients respectively (combining the results pertaining to days 6 and 7). Regarding the anti-SARS-CoV-2 antibody test results, patient 1 was tested negative for both IgG and IgM during the hospitalization. Patients 2 and 3 were tested negative for IgM throughout the period, and IgG antibody was positive on days 17 and 20, respectively.

This is the first case report on LAMP for diagnosing COVID-19 children. LAMP detects SARS-CoV-2 rapidly through an easy to interpret assay and is cost effective (Table 3) (3-7). Its sensitivity and specificity for diagnosing COVID-19 are thought to be high. In this study, the sensitivity was 63.6%, and specificity was 100% relative to the RT-PCR results

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during the hospitalization. Regarding samples of NPS, in patient 1, the positive LAMP results were consistent with the RT-PCR results on days 10, 13, and 17, and both tests were negative on day 20. In patient 2, the positive LAMP results were consistent with the RT-PCR results throughout hospitalization, except on day 1. Thus, the results of RT-PCR and LAMP using NPS were consistent, except at the very early stage of infection, suggesting that, LAMP would be less reliable than RT-PCR in very early stage of infection in school children. However, it would be suitable for use during follow-up. In patient 3, the sensitivity of LAMP was 33.3% relative to RT-PCR, suggesting that, LAMP would not be suitable for diagnosing COVID-19 in neonates.

The salivary RT-PCR results closely matched the NPS RT-PCR results in patients 1 and 2. With LAMP, salivary results reverted to negative earlier than the NPS results in the late stage of infection. These results suggest that saliva samples would be suitable for detecting SARS-CoV-2 on LAMP in school children in the early stage of infection, although that saliva would be less suitable than NPS in the late stage of infection. Saliva has been reported to be useful for RT-PCR to diagnose COVID-19 in adults. It is non-invasive and its collection is simple, with minimal personal protective equipment required by the person performing the sample collection due to minimal risk of exposure (8). In a study of South

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Korean children, RT-PCR results using saliva and NPS did not match, possibly due to different collection methods of saliva samples (9).

Although seropositivity rates with anti-SARS-CoV-2 antibody in adults are high from 14 days after symptom onset (10), antibody studies on asymptomatic cases are limited. In our two pediatric patients, IgG was detected 3 weeks after exposure to a COVID-19 patient.

A limitation of this study is that some salivary samples could not be tested due to the limited laboratory facilities at our hospital. Furthermore, the severity of symptoms has been reported to be proportional to the SARS-CoV-2 viral load (11); therefore, the test results may differ from our results in symptomatic patients with a high viral load. There were only three children in our study; therefore, larger studies are required to confirm the diagnostic accuracy of LAMP in COVID-19 children.

In conclusion, LAMP results appear to be consistent with RT-PCR results, except in the very early stage of infection in school children. LAMP would not be suitable for diagnosing COVID-19 in neonates. Salivary samples would be less sensitive than NPS samples for LAMP testing in the late stage of infection.

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Conflict of interest The authors have no conflicts of interest to declare.

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Table 1: Detailed clinical course and results of SARS-CoV-2[1] tests in the pediatric patients during hospitalization

Hospital day			-4	0	↓ Admitted 1	3	5	6	7	10	13	17	20	Discharged ↓ 21
Patient 1 8 years Male	Symptom				⇔ Loss of taste									
	RT-PCR[2]	NPS[4]			−	−		+		+	+	+	−	
		Saliva							+	+	−	+	−	
	LAMP[3]	NPS			−	−		−		+	+	+	−	
		Saliva							+	+	−	−	−	
Patient 2 6 years Male	Symptom						⇔ Transient cough							
	RT-PCR	NPS			+	+		+		+	+	+	+	
		Saliva	Exposure to father			+			+	+	+	+	+	
	LAMP	NPS			−	+		+		+	+	+	+	
		Saliva							+	+	+	−	+	
Patient 3 24 days on admission Female	Symptom									No symptoms				
	RT-PCR	NPS			−	+		+		+	−	−	−	
		Saliva								−	−	−	−	
	LAMP	NPS			−	+		−		−	−	−	−	
		Saliva								−				

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RT-PCR was performed at a local health center; therefore, we were unable to obtain information on the viral load. LAMP tests did not quantify RNA due to technical and equipment issues in our hospital.

1) SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; 2) RT-PCR, real-time reverse-transcription polymerase chain reaction; 3) LAMP, loop-mediated isothermal amplification; 4) NPS, nasopharyngeal swab

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Table 2: Patient characteristics

	Patient 1	Patient 2	Patient 3
Age on admission	8 years	6 years	24 days
Sex	Male	Male	Female
Complications	Nothing	AR[1)]	Nothing
Symptoms due to COVID-19[2)]	Loss of taste	Cough	Nothing
Laboratory studies on the date of diagnosis			
Leukocytes, $\times 10^3/\mu L$	7.83	10.7	11.41
Lymphocytes, $\times 10^3/\mu L$	3.63	5.73	7.42
Eosinophil, $\times 10^3/\mu L$	0.16	1.10	0.57
CRP[3)], mg/dL	0.07	0.04	0.02
D-dimer, μg/mL	0.2	N/A[4)]	0.5
LDH, International Unit/L	232	301	218
Creatinine, mg/dL	0.33	0.27	0.20
Ferritin, ng/mL	69.0	17.5	161.1

1) AR, allergic rhinitis; 2) COVID-19, coronavirus disease; 3) CRP, C-reactive protein; 4) N/A, not available

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Table 3. Comparison of RT-PCR[1)] and LAMP[2)]

RT-PCR	LAMP
4−8 hours until result	Within an hour until result
Requires expensive instrument Needs specialized thermal cyclers Bulky and cumbersome	Only a heat block is required Smaller, simpler, portable
Requires skilled technicians Significant technical labor	Not needed specific skill
	Diagnostic sensitivity > 95% Compared to RT-PCR results

1) RT-PCR, real-time reverse-transcription polymerase chain reaction; 2) LAMP, loop-mediated isothermal amplification

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