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The development of SARS-CoV-2 PCR testing methods at a designated medical institution for specific infectious diseases in Japan

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Abstract: Due to the coronavirus disease 2019 (COVID-19) pandemic, we have been conducting polymerase chain reaction (PCR) testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at this facility since March 2020. In the early days, the PCR testing system had limited capabilities, so testing had to be conducted manually and only a few tests were conducted. Moreover, we lacked sufficient experience to conduct PCR testing manually, so we struggled with the manual work, which required intense concentration, and we felt pressured not to make mistakes such as allowing contamination. Since we introduced upgraded equipment, new methods, and additional staff for testing and we cooperated with the clinical technologist on the night shift in the Emergency Department, we are currently able to conduct urgent PCR testing on more than 2,000 specimens per month 24 hours a day. We will continue to meet new needs for COVID-19 treatment with the cooperation of other departments.

***Keywords*:** SARS-CoV-2, PCR testing

Coronavirus disease 2019 (COVID-19) is a viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since February 2020, the Clinical Laboratory at our hospital (a designated medical institution for specific infectious diseases in Japan) has gradually enhanced its polymerase chain reaction (PCR) testing system for SARS-CoV-2 to meet the needs for COVID-19 treatment. Here, I would like to explain the development of our system and methods for SARS-CoV-2 PCR testing used at this hospital over the past two years.

Events related to the PCR testing system for SARS-CoV-2 over the past two years (Table 1)

In March 2020, the Clinical Laboratory introduced an in-hospital SARS-CoV-2 PCR testing system. In the early days, the testing system had limited capabilities, so testing had to be conducted manually and few tests were conducted. Since we introduced upgrading equipment, modified methods, and additional staff for testing, the number of tests gradually increased. In July, reagents for automated equipment were supplied. This allowed us to increase the number of tests. In August, urgent PCR testing started with the cooperation of the clinical technologist on the night shift in the Emergency Department. We conducted around-the-clock testing for patients who required emergency hospitalization or surgery.

In January 2021, we started PCR testing for recipients of full medical checkups. In March, we started PCR testing to detect the N501Y and E484K mutation of the SARS-CoV-2 spike protein in new patients with COVID-19. In June, we further added PCR testing to detect the L452R mutation of the SARS-CoV-2 spike protein. In July, we ended PCR testing by the Clinical Laboratory to detect mutant strains and we started the detection of mutant strains *via* Sanger sequencing by the Center for Clinical Sciences. In December, we started analyzing the entire genome with whole-genome sequencing.

As Figure 1 shows, the number of tests increased significantly compared to the early days, and we are able to effectively control infection and manage beds.

Changes in the PCR testing methods used in the Clinical Laboratory

From March to May 2020, we conducted PCR testing using primers and probes provided by the National Institute of Infectious Diseases in Japan (NIID-J) (*1*). Making all of the reagent adjustments from scratch took a lot of time and great effort. Therefore, we had a very hard time using that approach as a routine testing method. To prevent contamination and human error, we spent a lot of time managing the environment and training the staff for testing.

In June, we adopted a new PCR testing method using LightMix® Modular SARS and Wuhan CoV N-gene and E-gene (LightMix, Roche). The testing

Table 1. Main events related to the PCR testing system for SARS-CoV-2 over the past two years

February, 2020	Start to consider an in-hospital PCR testing at this facility's Clinical Laboratory
March, 2020	Start to assist with specimen management at this facility's fever clinic Start of in-hospital PCR testing by the Clinical Laboratory (about 5 specimens per day only on weekdays) Expansion to in-hospital PCR testing on weekends and holidays
April, 2020	Expansion to two assays on weekdays and 40 specimens per day
May, 2020	Start of preoperative PCR testing and prepartum PCR testing
August, 2020	Start of urgent PCR testing, 24 hours a day in cooperation with the clinical technologist on the night shift in the Emergency Department
September, 2020	End of outsourced PCR testing and start of PCR testing by the Clinical Laboratory
December, 2020	Start of PCR testing for all hospitalized patients
January, 2021	Start of PCR testing for patient visitors Start of PCR testing for recipients of full medical checkups
March, 2021	Start of PCR testing to detect N501Y and E484K mutations of the SARS-CoV-2 spike protein
June, 2021	Addition of PCR testing to detect the L452R mutation of the SARS-CoV-2 spike protein
July, 2021	End of PCR testing by the Clinical Laboratory to detect mutant strains and start of detection of mutant strains *via* Sanger sequencing by the Center for Clinical Sciences
December, 2021	Start of analysis of the entire genome *via* whole-genome sequencing by the Center for Clinical Sciences

Modified from Reference 2 (*2*).

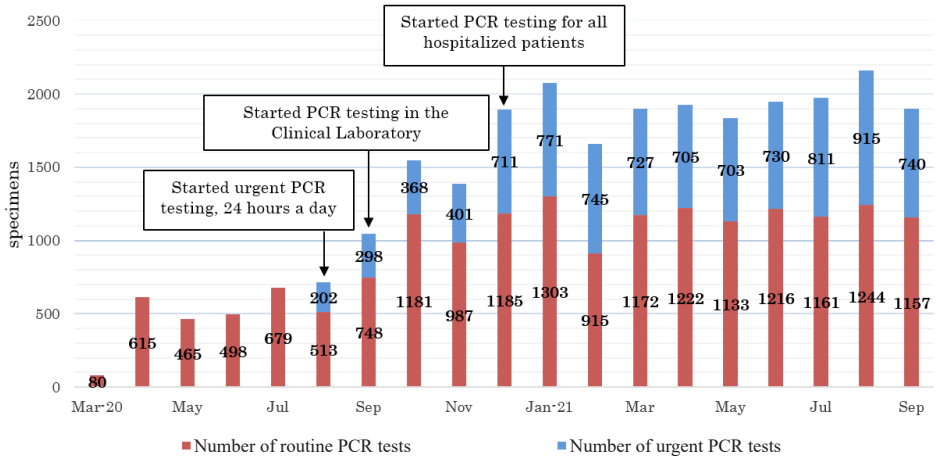

Figure 1. Number of tests conducted at this hospital. Modified from Reference 2 (*2*).

process was the same as before, but the PCR reaction time was much shorter (only one hour). The LightMix reagent adjustment was simpler than before. In addition, LightMix allowed us to measure the internal control and to prevent false negatives.

In July, we started using cobas SARS-CoV-2 (cobas, Roche), which can automatically conduct PCR testing on up to 94 specimens in one round. Therefore, this method greatly reduced the burden on the staff for testing.

In August, we adopted the Filmarray® Respiratory Panel 2.1 (Filmarray, BioMérieux), which takes only about an hour to complete. This allowed prompt reporting of each test result.

In December 2021, we introduced the Xpert Xpress SARS-CoV-2 'Cepheid' (Genexpert, Beckman Coulter), which we basically use for urgent PCR testing.

Currently, we have several other instruments and we are able to choose one based on the number of specimens and urgency.

Results of those changes

PCR testing consists of three major steps (1) extraction, (2) reagent preparation, and (3) amplification and detection. Before we started SARS-CoV-2 PCR testing, we used reagent kits for all PCR testing. Since the three processes were mostly automated before the start of SARS-CoV-2 PCR testing, we conducted the tests without highly specialized knowledge or skills. However, when the decision was made to conduct SARS-CoV-2 PCR testing in-house, we had to perform

(1) extraction and (2) reagent adjustment manually because no SARS-CoV-2 detection reagent kits were available for automated instruments. We did not have enough experience to conduct PCR testing manually. Therefore, we struggled with manual work, which required intense concentration, and we felt pressured to avoid contamination and mistakes. In April and May 2020 in particular, COVID-19 was spreading throughout Tokyo, and there was always a possibility that the staff for testing might be infected. Moreover, there was also a concern that we might face shortages of reagents and consumables for testing while the number of tests increased. However, we have gained valuable experience overcoming those difficulties.

As Figure 1 shows, we are able to test more than 2,000 specimens per month, using automated instruments to test large numbers of specimens. Moreover, we are able to conduct urgent PCR testing 24 hours a day in cooperation with the clinical technologist on the night shift in the Emergency Department.

We will continue to meet new needs for COVID-19 treatment with the cooperation of other departments.

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References

1. National Institute of Infectious Diseases. Manual for Pathogen Detection: 2019-nCoV Ver.2.9.1. *https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf* (accessed July 31, 2022). (in Japanese)
2. Motohashi A. The development of SARS-CoV-2 PCR testing methods in NCGM. In: The experiences and evidences of NCGM staff: New coronavirus infections COVID-19: 2020-2021 (National Center for Global Health and Medicine, eds). Public Relations and Planning Office, Department of Planning and Strategy, National Center for Global Health and Medicine, Tokyo, 2021; pp.109-113.

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