The temporal positivity rate of SARS-CoV-2 in different clinical samples

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research-note

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Abstract

The aim of this study is to evaluate the diagnostic value of different clinical samples from humans such as blood/serum, stool, and urine as compared to the routinely used nasopharyngeal swab samples for the detection of SARS-CoV2 in COVID-19 patients. We followed COVID-19 patients for three weeks and collected samples on three occasions that is, on the day of admission to the hospital (Day zero), after one week (Day-8), and after the second week (Day-15). The data shows that on the day of the admission of the patients, NPS has a 64% positivity rate, followed by stool, urine, and serum, 38%, 18%, and 17%, respectively. And we observed a nearly similar pattern of positivity rate in the subsequent week's samples.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) which is responsible for the coronavirus disease 2019 (COVID-19) has spread all over the world since its first outbreak in early December 2019 (1, 2). According to the COVID-19 dashboard by the Center for Systems Science and Engineering (CSSE) at John Hopkins University (JHU) (https://coronavirus.jhu.edu/map.html), as of August 24, 2022, 597,803,846 people were infected by the SARS-CoV2 virus, and a total of 6,458,430 deaths were recorded due to COVID-19 globally.

Laboratory diagnostic testing is one of the crucial measures for curbing the spread of COVID-19. However, the quality of a laboratory test result is dependent upon the type of specimen used and the choice of diagnostic methods (3, 4). Concerning the choice of method for the diagnosis of SARS-CoV-2, the world has agreed on using the Real-time reverse transcriptase–PCR (RT-PCR) as the standard method. Nevertheless, no consensus practical guideline recommending the appropriate type of specimen to be collected for the SARS-CoV-2 diagnosis is available (5). In our previous study which compared the positivity rate of the nasopharyngeal swab (NPS) and Saliva using 140 pairs of saliva-NPS samples, 92.14% (129/140) saliva samples tested positive for SARSA-CoV2 RNA whereas 57.14% (80/140) of NPS samples were positive, based on the finding we recommended saliva as a better alternative sample to NPS to diagnose COVID-19 patients (6). Here, as a continuation of our previous work (6), in this report, we present data on the positivity rate of SARS-CoV2 in blood/serum, stool, and urine samples with the commonly used NPS sample.

Methods

Clinical samples collection and preparation

All the samples were collected from symptomatic confirmed COVID-19 patients as described before in (6). The patients were admitted to St. Paul hospital five to seven days after they were confirmed positive by RT-PCR. NPS samples were collected using a viral transport medium (VTM). The first NPS, stool, blood, and urine samples were collected on the day of the patient's admission to the hospital (hereafter, day zero), followed by the collection of two (NPS, stool, blood, and urine) samples within a one-week interval

(on the 8th and 15th days). All the samples were transported from St. Paul hospital under an adequate cold chain of 4–8°C, kept refrigerated at 4°C at Armauer Hansen Research Institute, and were processed within 8–12 hours for RNA extraction. Blood samples were collected in serum separator tubes and centrifuged at 1500 rpm for 10 minutes at room temperature. Separated serum samples were aliquoted into cryotubes and stored at -80^{0}C for viral nucleic acid extraction. Urine samples were collected in leak-proof screw-capped tubes and transported at 4°C. Then the urine (20–40 ml) samples were centrifuged at 2500 x g for 15 minutes at 4°C. The pellets were resuspended in 2 ml phosphate-buffered saline (PBS) and stored at -80^{0}C for viral nucleic acid extraction. Stool samples were collected in a special cup (a wide-necked leak-proof screw-capped cup). Approximately 1 gram of stool was resuspended within 5 mL of normal saline, centrifuged at 10,000 rpm for 5 minutes, and 1 ml of the clarified supernatant was kept for viral detection.

RNA extraction and detection

Both viral RNA extraction and RNA detection by RT-PCR were processed exactly as it has been reported previously (6). Briefly, a volume of 200 µL samples of NPS, Stool, Serum, and Urine was used to extract viral nucleic acid (NA) using DAAN Gene Co., Ltd (Da An Gene Co., Ltd, of Sun Yat-Sen University, China) extraction and purification kit. In the 200 µL of samples, 50 µL proteinase K, and 200 µL lysis buffer was added, followed by heat inactivation of the lysed samples on a dry heat block at 72°C for 10 min, the addition of inhibitor remover, and subsequent washing. The NA was eluted in 50 µL molecular grade water. Finally, the SARS-CoV2 RNA was detected using the BGI Biotechnology (Wuhan) Co.Ltd, China detection kit (7).

Results

On the day of the admission of the patients (on Day-zero or within five to seven days after the patients were confirmed positive by RT-PCR), NPS has a 64% positivity rate, followed by stool, urine, and serum, 38%, 18%, and 17%, respectively. A similar pattern of positivity rate has been observed in the samples collected on the second and third week of the follow-up that is, NPS has the highest positivity rate followed by stool, urine, and serum (Table 1). Detailed data with the Ct values are presented in the Additional file1.

Table 1
SARS-CoV2 positivity rate over time (Day-0, Day-8, and Day-15) where 'N' represents the total number of patient samples, positives, and negatives processed.

Nasopharyngeal swab	Day-0 (N)	Day-0 Positivity rate	Day-8 (N)	Day-8 Positivity rate	Day-15 (N)	Day-15 Positivity rate
Total sample	152		70		14	
Positive	98	**64.47**	23	**32.86**	8	**57.14**
Negative	54	**35.53**	47	**67.14**	6	**42.86**
Serum						
Total sample	136		67		14	
Positive	23	**16.91**	8	**11.94**	1	**7.14**
Negative	113	**83.09**	59	**88.06**	13	**92.86**
Stool						
Total sample	45		28		5	
Positive	17	**37.78**	7	**25**	1	**20**
Negative	28	**62.22**	21	**75**	4	**80**
Urine						
Total sample	76		36		7	
Positive	14	**18.42**	4	**11.11**	1	**14.29**
Negative	62	**81.58**	32	**88.89**	6	**85.71**

Considering that sample collection began almost a week after the patients confirmed positive and were admitted to the hospital, we followed them for three consecutive weeks; this shows that there is shedding of the virus up to the fourth week. In addition, a clear pattern of viral load decrease over time has been observed particularly for NPS, serum, and Stool samples (Fig. 1).

Discussion

Our study shows that SARS-CoV2 RNA was detected in all types of clinical samples tested, including NPS, serum, stool, and urine. The highest positivity rate was detected in NPS followed by stool while the lowest was from the serum sample. Similarly, the lowest Ct value (meaning high viral load) was detected in the NPS and stool samples. This implies that NPS is the most appropriate clinical specimen(8), and stool is the most preferred specimen next to NPS. However, our data is in contrast to this study (8), which reported no detection of SARS-CoV-2 in urine and serum. Even though we reported a high positivity rate in NPS, there were few observations where samples from NPS detected no SARS-CoV2 while other

specimens turn out to be positive for the virus. This shows the need for testing specimens from multiple sites to improve the sensitivity and reduce false-negative test results (4). Because saliva is more sensitive than NPS and easy to collect for COVID-19 diagnosis (6, 9), the use of a combination of saliva, NPS, and stool sample greatly improves the sensitivity and reduces false negative test results of COVID-19 diagnosis.

Declarations

Ethics approval and consent to participate: The study is approved by the Armauer Hansen Research Institute/ALERT Ethics Review Committee. And informed consent was obtained from all study participants. All methods were performed in accordance with the guidelines and regulations stipulated in the Ethiopian national comprehensive COVID-19 management handbook.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are available (Additional file1).

Competing interests: The authors declare no competing interests.

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Author contributions

Conceived the project idea: AM, GTB, AA, AdM, and LY. Conducted laboratory work: FA, ESK, DHA, TS, DAT, GA, AT, AH, GB, BT, and MY. Data analysis: FA, GTB, AA, AdM, and LY. Wrote – the original draft: GTB, FA, AM, AA, and AdM. Writing – review & editing: GTB, FA, AM, GTB, AA, AdM, and LY.

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Figures

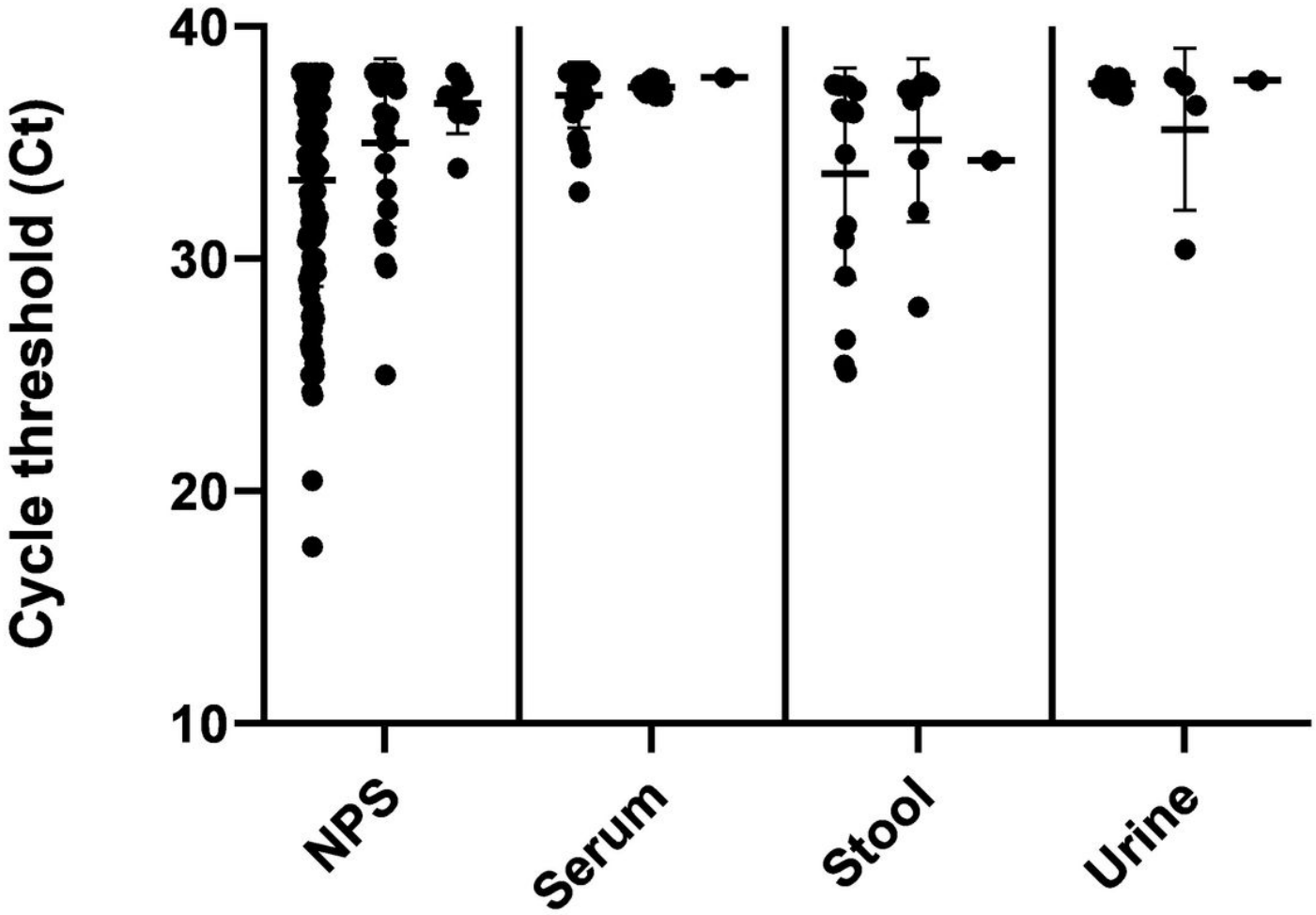

Figure 1

Temporal shedding of SARS-CoV2 in NPS, Serum, Stool, and Urine samples as expressed by the Ct values. The nested scatter plots of each clinical sample from left to right represent positive data points from Day-0, Day-8, and day-15. All the positive data points can be obtained from Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx