Clinical Infectious Diseases

BRIEF REPORT

Testing for SARS-CoV-2: Can We Stop at 2?

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The COVID-19 epidemic requires accurate identification and isolation of confirmed cases for effective control. This report describes the effectiveness of our testing strategy and highlights the importance of repeat testing in suspected cases in our cohort.

Keywords. SARS-CoV-2; COVID-19; diagnosis; polymerase chain reaction; testing.

Since the current outbreak of coronavirus disease 2019 (COVID-19) was first reported from Wuhan, China, on 31 December 2019, more than 60 countries have reported cases [1]. It has resulted in approximately 90 000 cases and about 3000 deaths. In several countries, the number of cases has escalated rapidly. Strategies to contain the disease include active case-finding and isolation of suspected or confirmed cases, which relies on accurate and early diagnosis to reduce the risk of undiagnosed cases infecting others [2]. We describe the outcomes of our testing strategy for COVID-19 at the National Centre for Infectious Diseases, Singapore (NCID).

METHODS

Since the start of the COVID-19 epidemic, all individuals in Singapore who met the criteria for suspected COVID-19 infection were admitted to airborne-infection isolation rooms in public hospitals. The criteria included travelers with acute respiratory symptoms from Hubei province, China, or close contact with a confirmed case of COVID-19 infection. Clinical specimens for testing include nasopharyngeal swabs, sputum, and stool samples if diarrhea is present. A minimum of 2 specimens were collected at least 24 hours apart to account for disease progression and to increase yield [3]. The diagnosis of COVID-19 infection was confirmed through reverse transcription–polymerase chain reaction (RT-PCR) testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A laboratory-developed test targeting the *N* and *ORF1ab* genes was used at the start of the outbreak in Singapore in late January 2020. From 6 February 2020, a commercial assay was used. The details of the assays are described in Supplementary Appendix 2. Data collection was approved under the Infectious Diseases Act [4].

RESULTS

As of 29 February 2020, 102 cases were detected locally and 72 were managed at NCID. Two cases were asymptomatic and were excluded from subsequent analysis. Among 70 patients, the median time from symptom onset to presentation at NCID was 5 days (range, 1–24 days). The median time from symptom onset to the first positive test was 6 days (range, 1–24 days), and the median time from presentation to the first positive test was 0 days (ie, the same day of presentation at NCID; range, 0–7 days). Patients who required more than 1 test for SARS-CoV-2 had a median of 5.5 days of symptoms (range, 2–22 days).

Sixty-two of 70 patients (88.6%) had SARS-CoV-2 detected from their first clinical specimen collected on the first day of admission. These were all nasopharyngeal specimens. In 5 (7.1%) patients, SARS-CoV-2 was detected on the second clinical specimen, which was collected 24 hours after the first, increasing the yield of our strategy to 95.7%. The remaining 3 (4.3%) patients (A, B, and C) needed more than 2 samples to confirm the diagnosis. The number of cases who tested positive by day of symptom onset is shown in Figure 1A. The clinical manifestations along with testing dates and results are summarized in Figure 1B.

Patient A was a 35-year-old male traveler from Wuhan, China. His symptoms of fever, dry cough, sore throat, and diarrhea started on 24 January 2020. He presented to NCID on the same day. Chest radiograph showed patchy airspace opacification in the right lower zone. Polymerase chain reaction from nasopharyngeal swabs on 25 and 26 January did not detect SARS-CoV-2. The patient tested positive on the third nasopharyngeal specimen on 27 January.

Patient B was a 41-year-old female traveler from Wuhan, China. Her symptoms of fever and dry cough started on 28 January 2020. Chest radiography showed patchy consolidation in the left middle zone. Her fever resolved on 29 January. SARS-CoV-2 was not detected on her nasopharyngeal specimens on 28, 29, and 31 January and 1 February. Multiplex PCR for respiratory viruses using a commercial assay was negative. As she was clinically well, a decision was made to resume testing after 7 days of symptom

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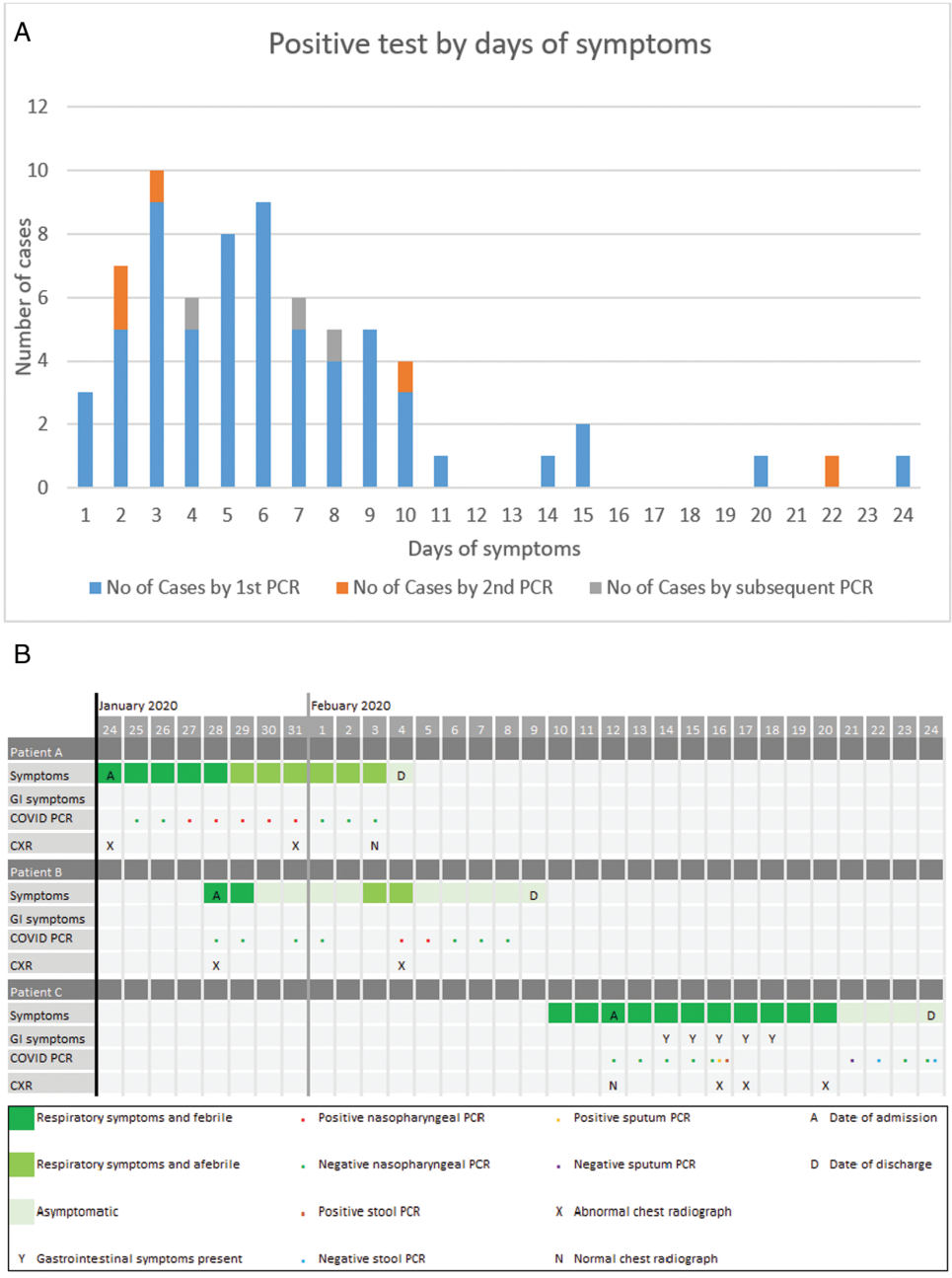

Figure 1. *A*, Number of cases who tested positive for SARS-CoV-2 by days of symptoms. *B*, Clinical course of 3 patients who were diagnosed after more than 2 PCR tests. Abbreviations: CXR, chest X-ray; COVID, coronavirus disease; GI, gastrointestinal; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

onset, extrapolating from the viral kinetics in SARS where viral loads are highest in the second week of illness [5]. She remained in airborne-infection isolation during this period. Her fifth nasopharyngeal swab on 4 February detected SARS-CoV-2.

Patient C is a Singaporean male with no history of travel to China. He was a close contact of a local confirmed case. He had fever and cough that started on 10 February. He was admitted on 12 February. There was no abnormality on chest radiograph on admission. He had persistent fever during admission. His nasopharyngeal swabs were negative for SARS-CoV-2 on 12, 13, 14, and 15 February. Multiplex PCR for respiratory viruses was negative. He started to have productive cough and mild diarrhea late on 15 February. On 16 February, his sputum and stool samples tested positive for SARS-CoV-2. The result from the nasopharyngeal specimen on 16 February, his fifth, were negative for SARS-CoV-2.

DISCUSSION

Accurate and reliable diagnosis of COVID-19 infections remains the cornerstone of the public health strategy for disease containment. Earlier reports described high viral loads in upper respiratory specimens soon after symptom onset, which peaked in the first few days before declining [6, 7]. Our testing strategy was able to detect 67 out of 70 (95.7%) cases, as patients presented to our center at a median of 5 days from symptom onset. This possibly contributed to our high detection rates.

However, it is important to continue sampling in highly suspicious cases as initial results can be negative. Patients B and C had persistently negative tests from upper respiratory samples until days 8 and 7 of symptom onset, respectively. Additionally, patient C's nasopharyngeal specimen remained negative despite positive stool and sputum samples. This could possibly be due to natural history of disease, intermittent viral shedding, low viral load in the upper respiratory tract when the disease is predominantly in the lower tract, and variations in sample collection technique. The virus may be detected in stool samples in 50% of patients with COVID-19 infection [7]. In addition to testing respiratory samples, testing stool samples, especially in patients with gastrointestinal symptoms, may increase diagnostic yield.

We agree with World Health Organization and US Centers for Disease Control and Prevention recommendations that lower respiratory tract samples should be tested when possible [3, 8]. In addition, adjunctive investigations, in particular, computed tomography (CT) scans of the thorax, could further enhance the sensitivity of case detection [9]. Serological tests are unlikely to be useful in the early diagnosis of COVID-19 infection. In a cohort of 173 patients tested for antibodies against SARS-CoV-2 using enzyme-linked immunosorbent assay, only 36% had detectable antibodies in the first week of illness [10].

In light of these outliers, we have developed a decision-making matrix, factoring in clinical, laboratory, and epidemiological factors to facilitate de-isolation and discharge of individuals with suspected COVID-19 [11]. To date, none of the discharged patients have re-presented with COVID-19.

In our cohort, active case detection of those with suspected COVID-19 in the early course of illness combined with daily sequential sampling of upper respiratory tract specimens over 2 days has allowed for the detection of the majority of COVID-19 cases. However, added caution should be taken in interpreting negative results in patients with suspicious clinical or epidemiological features. A decision-making matrix or adjunctive CT scans of the thorax could be implemented to guide decisions on further repeat testing and de-isolation in such patients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Note

Potential conflicts of interest. T. B. is a co-inventor of the Fortitude Kit, (patent pending) for detecting a virus 102020012005. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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