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

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Abstract:

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 suspect cases in our cohort.

Introduction:

Since the current outbreak of coronavirus disease (COVID-19) was first reported from Wuhan, China, on 31 December 2019, more than 60 countries have reported cases.[1] It had resulted in approximately 90,000 cases and about 3,000 deaths. In several countries, the number of cases had escalated rapidly. Strategies to contain the disease include active case-finding and isolation of suspect or confirmed cases, hinging 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 suspect criteria for 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 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 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 February 6, 2020, a commercial assay was used. The details of the assays are described in the Supplement.

Data collection was approved under the Infectious Diseases Act. [4]

Results:

As of February 29, 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, i.e. the same day of presentation at NCID (range 0-7 days). Patients who required more than 1 test for SARS-CoV-2 had median 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 five (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 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 January 24, 2020. He presented to NCID on the same day. Chest radiograph showed patchy airspace opacification in the right lower zone. PCR from nasopharyngeal swabs on January 25 and 26 did not detect SARS-CoV-2. He tested positive on the 3rd nasopharyngeal specimen on January 27.

Patient B was a 41 year-old female traveler from Wuhan, China. Her symptoms of fever and dry cough started on January 28, 2020. Chest radiography showed patchy consolidation in the left middle zone. Her fever resolved on January 29. SARS-CoV-2 was not detected on her nasopharyngeal specimens on January 28, 29, 31 and February 1. 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 onset, extrapolating from viral kinetics in SARS where viral loads were highest in the 2nd week of illness.[5] She remained in airborne infection isolation during this period. Her 5th nasopharyngeal swab on February 4 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 February 10. He was admitted on February 12. 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 February 12, 13, 14, and 15. Multiplex PCR for respiratory viruses was negative. He started to have productive cough and mild diarrhea late on February 15. On February 16, his sputum and stool sample tested positive for SARS-CoV-2. The result from the nasopharyngeal specimen on February 16, his 5th, remained undetected.

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 till days 8 and 7 of symptom onset respectively. Additionally, patient C's nasopharyngeal specimen remained negative despite positive stool and sputum samples. These could possibly be due to natural history of disease, intermittent viral shedding, low viral load in 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 Center for Disease Control recommendations that lower respiratory tract sample should be tested when possible.[8][9] In addition, adjunctive investigations, in particular, computed tomography (CT) scans of the thorax could further enhance the sensitivity of case detection.[10] 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.[11]

In light of these outliers, we have developed a decision making matrix, factoring clinical, laboratory and epidemiological factors to facilitate de-isolation and discharge of suspects.[12] To date, none of the discharged suspects have re-presented with COVID-19.

In our cohort, active case detection of suspects in the early course of illness combined with daily sequential sampling of upper respiratory tract specimens over 2 days has allowed for 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.

Conflicts of interest:

Timothy Barkham is a co-inventor of the Fortitude Kit, patent pending for: detecting a virus 102020012005. No other author has conflicts of interest declared.

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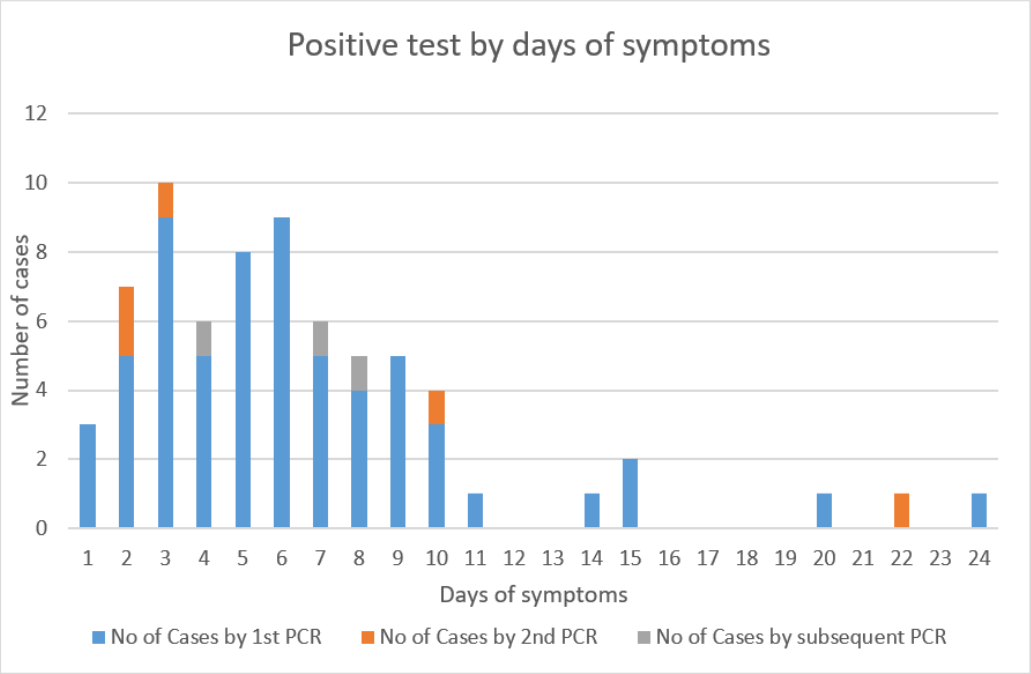
FIGURE LEGENDS

Figure 1a: Number of cases that tested positive for SARS-CoV-2 by days of symptoms.

Figure 1b: Clinical course of 3 patients that were diagnosed after more than 2 PCR tests



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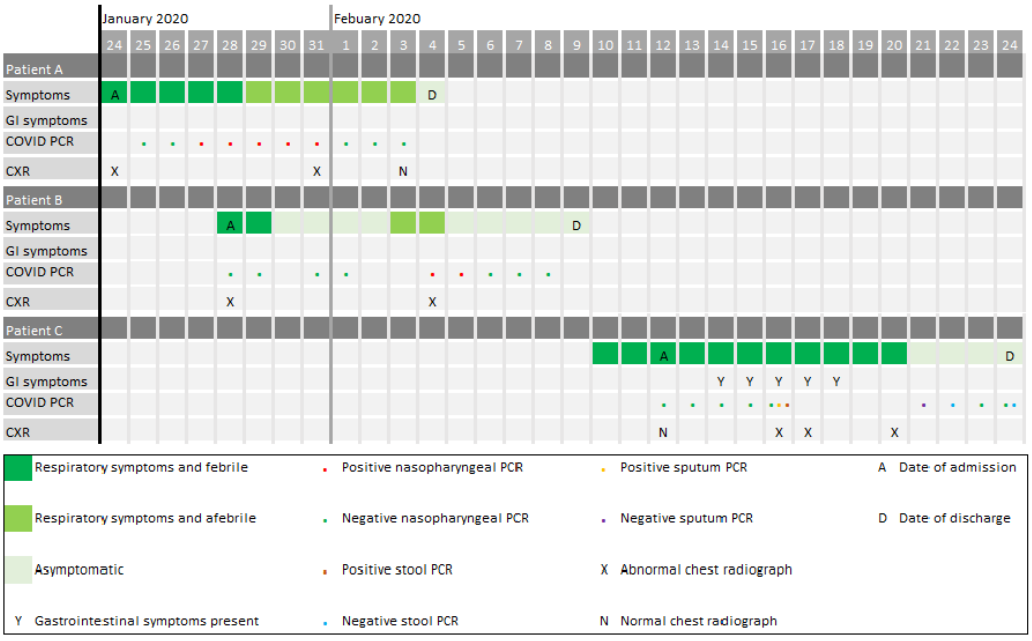

Figure 1b: Clinical course of 3 patients that were diagnosed after more than 2 PCR tests