An understanding of discordant SARS-CoV-2 test results: an examination of the data from a central Auckland laboratory

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ABSTRACT

AIM: The diagnostic sensitivity of the SARS-CoV-2 real time reverse transcription polymerase chain reaction (RT-PCR) test has not been determined. This has led to a degree of uncertainty in the interpretation of results, particularly in patients tested repeatedly. The aim of this study was to explore the characteristics of patients who initially tested negative, and subsequently tested positive for SARS-CoV-2.

METHODS: This retrospective observational study utilised data from the LabPlus Virology laboratory, Auckland City Hospital, to identify cases (hospital and community) with initial negative and subsequent positive SARS-CoV-2 RT-PCR results. Their clinical and laboratory characteristics were summarised.

RESULTS: From 1 February to 13 April a total of 20,089 samples were received for SARS-CoV-2 testing. Of 2,011 samples from patients with multiple tests, 25 samples were positive. Nine samples were from patients who initially tested negative then tested positive. Reasons for the initial negative test results, which were all from upper respiratory tract samples, included pre-symptomatic presentation or late presentation. All patients had significant risk factors and ongoing or evolving symptoms, which warranted repeat testing.

CONCLUSION: Few patients had discordant test results for SARS-CoV-2 RT-PCR. For patients who have a significant risk factor and a negative test result, repeat testing should be performed.

SARS-CoV-2 is the causative agent of Coronavirus Disease 2019 (COVID-19). The SARS-CoV-2 pandemic has infected over seven million people worldwide (data accessed on 8 June), with 1,504 cases in New Zealand.[1] Essential to halting the spread of SARS-CoV-2 has been the early detection and isolation of cases.[2,3] This is possible due to timely and reliable diagnostic testing.

Real time reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 gene targets is the preferred test for diagnosing acute infection.[3,4] The RT-PCR SARS-CoV-2 test was not extensively validated prior to widespread use, due to the urgent need for rapid deployment of a diagnostic test for COVID-19. As a consequence, the diagnostic performance of the test including the diagnostic sensitivity and specificity has not been well defined.[5] In contrast, the analytical sensitivity of the RT-PCR test has been established (limit of detection 500 copies per reaction or lower), and is comparable across the assay protocols and gene targets most commonly used worldwide.[6] The analytical sensitivity of the in house SARS-CoV-2 RT-PCR assay at the LabPlus Virology laboratory, Auckland City Hospital, is 10 copies per reaction.

Analytical sensitivity describes the smallest quantity of a target detectable by a test. Diagnostic sensitivity is the ability of a test to detect persons with the disease, for which a comparator test is required.[7] Currently for SARS-CoV-2, RT-PCR is the recommended test and therefore diagnostic sensitivity is difficult to establish.[3]

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There are case reports of patients who were tested multiple times for SARS-CoV-2 with discordant negative/positive results.[8–10] Fang et al report 15 patients who had radiological changes on chest computerised tomography but initial negative RT-PCR tests, although the details of sample type and symptoms for these patients are not clear.[11] Arons et al conducted point prevalence surveys seven days apart with residents of nursing care facilities. Upper respiratory tract samples were collected; 24 out of 76 residents tested negative initially then positive seven days later.[12] West et al speculate on the potential impact of the falsely reassuring interpretation of negative results, depending on varying scenarios of SARS-CoV-2 prevalence and extent of testing.[13] This uncertainty has led to a call for clear and accurate test interpretation.[13]

At time of writing, in New Zealand the focus remains on ensuring widespread community testing of symptomatic people and targeted sentinel surveillance of asymptomatic people to detect low-level community transmission. For this reason, it is essential to understand the various factors that could lead to a negative SARS-CoV-2 test result in an individual who subsequently tests positive for the virus. This information can then be used to support both diagnostic algorithms and public health investigations in high-risk settings.

This retrospective observational study aims to describe the characteristics of patients who initially tested RT-PCR negative before testing positive for SARS-CoV-2.

Method

Setting

This study was conducted at the LabPlus Virology laboratory, Auckland City Hospital. The laboratory processes hospital and community samples (from general practice and community-based assessment centres) for SARS-CoV-2.

The RT-PCR test uses primers and probes specific for the envelope protein gene (E gene) of SARS-CoV-2, based on the protocol published by Charité Virology, Berlin.[14]

Data collection

A database search of routinely collected laboratory data was conducted. Samples registered for the SARS-CoV-2 test from 1 February 2020 to 13 April 2020 were included. Data collected included the national hospital index number, age, gender, sample type, location of sample collection, date of collection and test result. Patients who had more than one sample tested, with at least one positive result were identified. Further information was sought for patients who had a negative test result followed by a positive result.

Information on whether the case was a close or causal contact was obtained from the Auckland Regional Public Health referral form, according to the Ministry of Health contact tracing definitions.[15] The clinical records and public health contact tracing records were reviewed to provide details about the timing and nature of the symptoms at the time of each specimen collection. Swabs were listed as nasopharyngeal, throat or upper respiratory tract (URT) if not differentiated further. The cycle threshold (CT) value for positive samples was obtained from laboratory records. The CT value is the point at which the exponential amplification of a gene target crosses a threshold value in the RT-PCR reaction. A high CT value occurs when there is a small amount of the gene target in the initial sample (ie, low viral load).

Data analysis

Anonymised data were used for analysis. Data analysis was conducted using Microsoft Excel (Microsoft Office 2010).

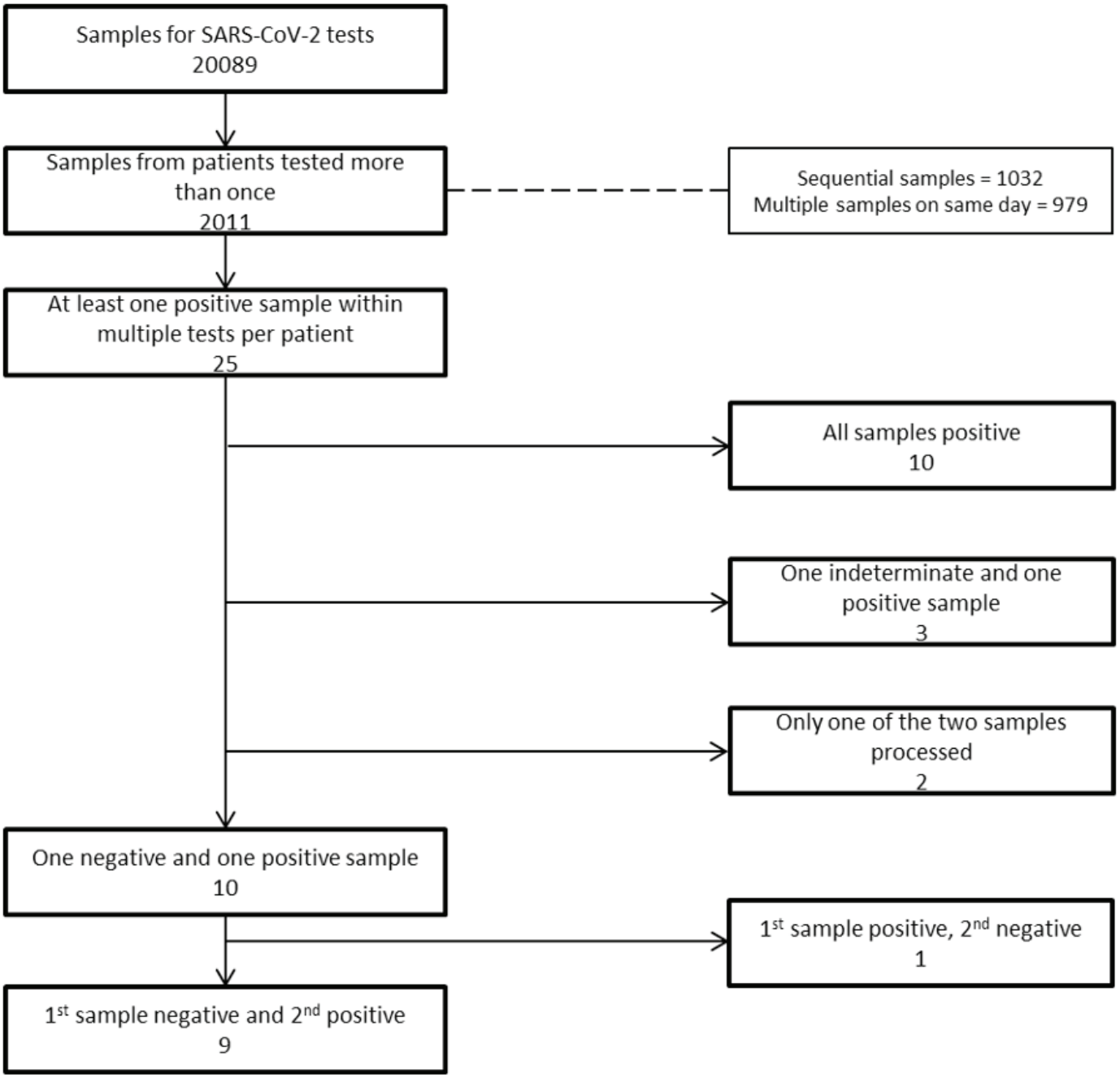
Ethics

This study received exemption from ethics review from the Auckland District Health Board Research Office.

Results

During the study period 20,089 samples were received for SARS-CoV-2 testing (Figure 1). There were 2,011 (10%) samples from patients who had been tested more than once. Roughly half of these (979, 49%) were multiple samples sent on the same day, whereas the remaining 1,032 (51%) were multiple samples sent on different days. There were 25 (1.2%) samples that had at least one positive result among the samples sent for that patient. Of these, nine (36%) were from patients who initially tested negative and subsequently positive for SARS-CoV-2.

Figure 1: Number of samples tested by RT-PCR for SARS-CoV-2 from 1 February to 13 April.



The three indeterminate samples produced inconclusive low take-off signals late in the RT-PCR reaction. They were reported as indeterminate with a comment asking for another sample to be sent for testing.

Further data were collected on the nine patients who initially tested negative and subsequently tested positive. These data are summarised in Table 1.

Where available, the clinical details for the first and second sample for each patient have been provided. Where the site of an upper respiratory tract specimen was not clearly labelled as nasopharyngeal or oropharyngeal, the sample type has been recorded as an upper respiratory tract (URT) swab.

Cases 1 and 2 were admitted to hospital due to COVID-19, with features of lower

Table 1: Characteristics of the nine patients who tested negative and then positive for SARS-CoV-2.

Case	Risk factor	Symptoms	Days from symptom onset to first sample	Days from symptom onset to second sample	CT E gene for positive sample	Sample type
1	Overseas travel	Headache, coryza, dry cough, dyspnoea	15	16	26.18	NPS, then Sputum
2	Close contact	1st sample: fever, myalgia 2nd sample: dyspnoea, haemoptysis	6	7	31.50	URT x 2
3	Close contact	1st sample: no clinical symptoms 2nd sample: cough, dyspnoea, sore throat	-2	6	20.93	URT x 2
4	Close contact	1st sample: no clinical symptoms 2nd sample: cough, sore throat	-10	0	21.41	URT x 2
5	Overseas travel	Fever, sore throat, anosmia, myalgia, arthralgia	0	6	21.50	URT x 2
6	Close contact	1st sample: cough, sore throat 2nd sample: fever	0	6	18.76	URT x 2
7	Close contact	Cough, fever	1	9	26.83	URT x 2
8	Close contact	Cough, sore throat, (known asthmatic)	4	12	27.7	URT x 2
9	Overseas travel	1st sample: cough, dyspnoea. 2nd sample: worsening symptoms	9	13	28.6	URT x 2

NPS= nasopharyngeal swab, URT = upper respiratory tract swab, CT = cycle threshold, E gene = envelope gene, RT-PCR gene target for SARS-CoV-2.

respiratory tract infection (both had chest x-ray abnormalities consistent with infection). A sputum sample obtained from case 1 was positive. There was no lower respiratory tract sample from case 2, and the second URT sample sent detected the highest CT value among this set of cases.

Cases 3 and 4 were asymptomatic when they tested negative. They subsequently developed symptoms and tested positive.

Cases 5, 6 and 7 presented on, or the day after, symptoms started. Two were close contacts of confirmed cases and one had recently returned from overseas.

Case 8 had respiratory symptoms on a background of previously diagnosed asthma. There were eight days between collection of the samples.

Case 9 reported subjective fever on the day of return from overseas. Cough and

dyspnoea developed, and nine days after the episode of fever the first test was performed. The second sample was taken four days after worsening of symptoms. Only upper respiratory tract samples were obtained.

Discussion

In the 10-week period from 1 February to 13 April, 20,089 samples were received for SARS-CoV-2 testing. Only nine out of 2,011 (0.4%) samples from patients who had multiple tests had an initial negative test followed by a positive RT-PCR for SARS-CoV-2. However, this likely represents the low prevalence of SARS-CoV-2 within the dataset, as only 25 (1.2%) samples were from patients with at least one positive result. This observational study may not be representative of the true proportion of confirmed cases that initially test negative because all patients were not tested sequentially in a systematic manner; the decision to repeat testing was at the clinician's discretion.

Review of the pattern of presentation provides a reasonable explanation for four of the nine cases. Late presenters; cases 1 and 2 had lower respiratory tract infection and negative upper respiratory tract samples. Pre-symptomatic; cases 3 and 4 were tested too early following exposure.

However, for the other five cases we can only postulate a reason for the discordant results. Cases 5 and 6 tested negative on the day of symptom onset, and positive six days later. The incubation period of SARS-CoV-2 is typically five to six days.[16] Both cases had high viral loads consistent with new onset illness rather than late presentation. Cases 8 and 9 had positive results late after the onset of symptoms with moderate viral loads also suggesting more recent onset of illness. Prolonged viral shedding has also been described for up to 10 days in mild cases.[17] We cannot comment on the accuracy of the clinical information recorded in this retrospective study. Case 7 tested negative the day after symptom onset, and positive eight days later. The initial negative result could be due to inadequate sample quality or alternative causes for the non-specific symptoms of COVID-19. Appropriate sampling technique is essential in order to obtain sufficient cellular material to detect SARS-CoV-2.[18]

Late presentation impacts on the ability of SARS-CoV-2 to be detected from upper respiratory tract samples. Studies have shown that SARS-CoV-2 can be detected from both upper and lower respiratory tract samples, although sputum samples have a higher viral load.[19,20] COVID-19 often features a deterioration of symptoms typically in the second week of illness.[21] Patients presenting later in the course of COVID-19 with lower respiratory tract symptoms should have lower respiratory tract specimens taken for SARS-CoV-2.[2,4] This is illustrated via cases 1 and 2, who were both hospitalised with COVID-19. Only upper respiratory tract samples were obtained from case 2, with a high CT value (therefore low viral load) detected on the second sample. This likely reflects the declining viral load in the upper respiratory tract, and therefore the lower probability of detecting the virus in such samples.[18] The nasopharyngeal swab from case 1 tested negative, whereas the sputum sample tested positive, as would be expected.

The role of testing asymptomatic and pre-symptomatic patients is unclear. There are reports in the literature of small numbers of asymptomatic patients testing positive, although more commonly these patients go on to develop symptoms.[10,22] A point prevalence study conducted in nursing care facility residents found that of 48 out of 76 residents testing positive, 27 (56%) were asymptomatic at the time of testing but 24 (89%) subsequently developed symptoms (median time to onset was 4 days).[12] A study in European citizens repatriated from Wuhan found that 335 out of 337 were asymptomatic, and all 337 tested negative on day 0 and day 5 after return suggesting that asymptomatic disease was uncommon.[23] Kimball et al describe 23 aged-care facility residents, only three of whom were asymptomatic and tested positive.[24]

The majority of studies report that the viral load is highest, and consequently CT value lowest, in the first few days following symptom onset.[19,20,25,26] Contrastingly, Zheng et al report that in respiratory samples (saliva after deep cough and sputum) peak viral load in mild illness occurred around day 10 and in severe illness around day 15.[27] A study of patients who had repeated RT-PCR tests found that the median time

from symptom onset to the first positive result was six days.[28]

The limitations of this study include that it is a single-centre study and despite capturing all tests conducted in the 10-week period, only nine patients had negative followed by positive results. This retrospective analysis relied on routinely collected data and no further interviews of the case were conducted. This study relied upon the requesting clinicians' decision to repeat the SARS-CoV-2 RT-PCR test. All the cases in this study had a significant exposure history (close contact of a case or recent overseas travel) which is why repeat testing was performed. In the absence of significant risk factors cases with discordant results may have been missed if they did not have repeat testing. However given the broad case definition for testing,[2] in keeping with New Zealand's approach to test widely for SARS-CoV-2, it is unlikely that patients with significant risk factors and ongoing or evolving symptoms would not have had repeat testing or be picked up in subsequent contact traces.

In conclusion, this study found small numbers of patients with discordant RT-PCR SARS-CoV-2 results. Of the 2,011 samples from patients who had multiple tests, only 25 were positive, and nine of these were from patients that tested negative and then positive by RT-PCR for SARS-CoV-2. These discordant results were seen in patients presenting late in the course of their illness or following exposure but before the onset of symptoms. The nonspecific nature of COVID-19 symptoms means that in some cases with an initial negative result, where there was a clear exposure history, repeat testing is required. Patients presenting late in the illness, with severe symptoms should have lower respiratory tract samples collected. Where there is high clinical suspicion that the person is infected with SARS-CoV-2 and the initial RT-PCR test result is negative, repeat testing should be performed.[28]

Competing interests:
Nil.

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