

Title: Limited clinical utility of early repeat RT-PCR testing for SARS-CoV-2

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Abstract: A review of 1,391 patients with repeat SARS-CoV-2 RT-PCR testing performed within 7 days over a 7-week period at Melbourne teaching hospital demonstrated that just 25/1,391 (1.8%) patients had discordant (initial negative and subsequent positive) SARS-CoV-2 RT-PCR results. All patients with discordant results had at least one epidemiological risk factor for COVID-19.

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The gold standard diagnostic assay for severe acute respiratory syndrome coronavirus (SARS-CoV-2) is reverse-transcription polymerase chain reaction (RT-PCR). The analytical performance of SARS-CoV-2 RT-PCR tests has been well described (1), however there are limited data regarding the clinical (cf. analytical) performance of RT-PCR (2). Pre-analytical factors such as timing of illness, anatomical sample site and sample collection may impact on clinical performance.

To further understand the clinical performance of SARS-CoV-2 RT-PCR, we assessed the frequency and characteristics of discordant SARS-CoV-2 RT-PCR results in Melbourne, Victoria, Australia. Between 1st June and 21st July 2020 Melbourne was experiencing a “second wave” of COVID-19. A total of 15,358 SARS-CoV-2 RT-PCR tests were performed in our laboratory at the Royal Melbourne Hospital, Melbourne, Australia on 12,569 unique patients (Table 1), using previously described methods [3]. Of these 15,358 SARS-CoV-2 RT-PCR tests, 12,215 (79.5%) were performed on patients attending health services for SARS-CoV-2 testing, with the remainder performed on symptomatic or asymptomatic healthcare workers. A risk-based approach to screening was undertaken; all patients requiring hospital

admission who met the Victorian Department of Health and Human Services case definition for suspected COVID-19 [4] required two consecutive negative combined deep nasal and oropharyngeal swabs, or one negative combined deep nasal and oropharyngeal swab and one negative sputum or tracheal aspirate prior to standing down transmission-based infection control precautions (Supplementary Table 1).

Of the 12,569 patients tested during the study period, 2,218 (17.6%) underwent repeat testing. Repeat testing was performed within 7 days in 1,391 patients (Supplementary Figure 1). Of these, 25/1,391 (1.8%) had initial negative results followed by a subsequent positive result within 7 days. All 25 patients had at least one epidemiological risk factor for COVID-19 (known contact with a confirmed COVID-19 case; contact with a confirmed outbreak within the healthcare or residential setting; or occupational healthcare exposure) (Table 1). Although a detailed assessment of epidemiological risk factors could not be performed for all individuals tested during the study period for comparison, this finding is notable given the relatively low prevalence of COVID-19 in Victoria during this period (peak rate of infection 54.2 per 100,000 population [5]). Importantly, 12/25 (48%) patients were asymptomatic at the time of their initial sample, which was collected in the setting of contact with a confirmed COVID-19 case, outbreak or asymptomatic healthcare worker surveillance; suggesting the initial swab may have represented the patients' incubation period after a known exposure, rather than a false negative result. Of 1,105 patients with a repeat SARS-CoV-2 RT-PCR within 24 hours of their initial test, only one patient returned a subsequent positive result. This patient had a sputum collected as their subsequent sample after a negative nasopharyngeal swab, which has been demonstrated to be a more sensitive sample type for SARS-CoV-2 [6].

Our observations in a low-prevalence setting suggest that progression from a negative to a positive SARS-Co-2 RT-PCR result within 7 days was uncommon and was not observed outside of well-defined epidemiological risk groups. These findings suggest that a risk-based approach to repeat testing for SARS-CoV-2 based on epidemiological risk factors may safely reduce the need for repeat sampling. These data informed a change in hospital policy such that repeat swabs were not routinely required for hospitalized patients except in defined epidemiological risk groups. We suggest that in low-prevalence settings, a risk-based approach to screening may result in improved patient flow in healthcare settings, reduced use of personal protective equipment, reduced patient discomfort and conservation of limited testing reagents.

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Conflicts of interest: The authors declare no conflicts of interest.

Ethics approval: This study was undertaken as part of routine validation and quality assurance activities related to the introduction of new in-vitro diagnostic devices, approved by the Melbourne Health HREC (QA2019134).

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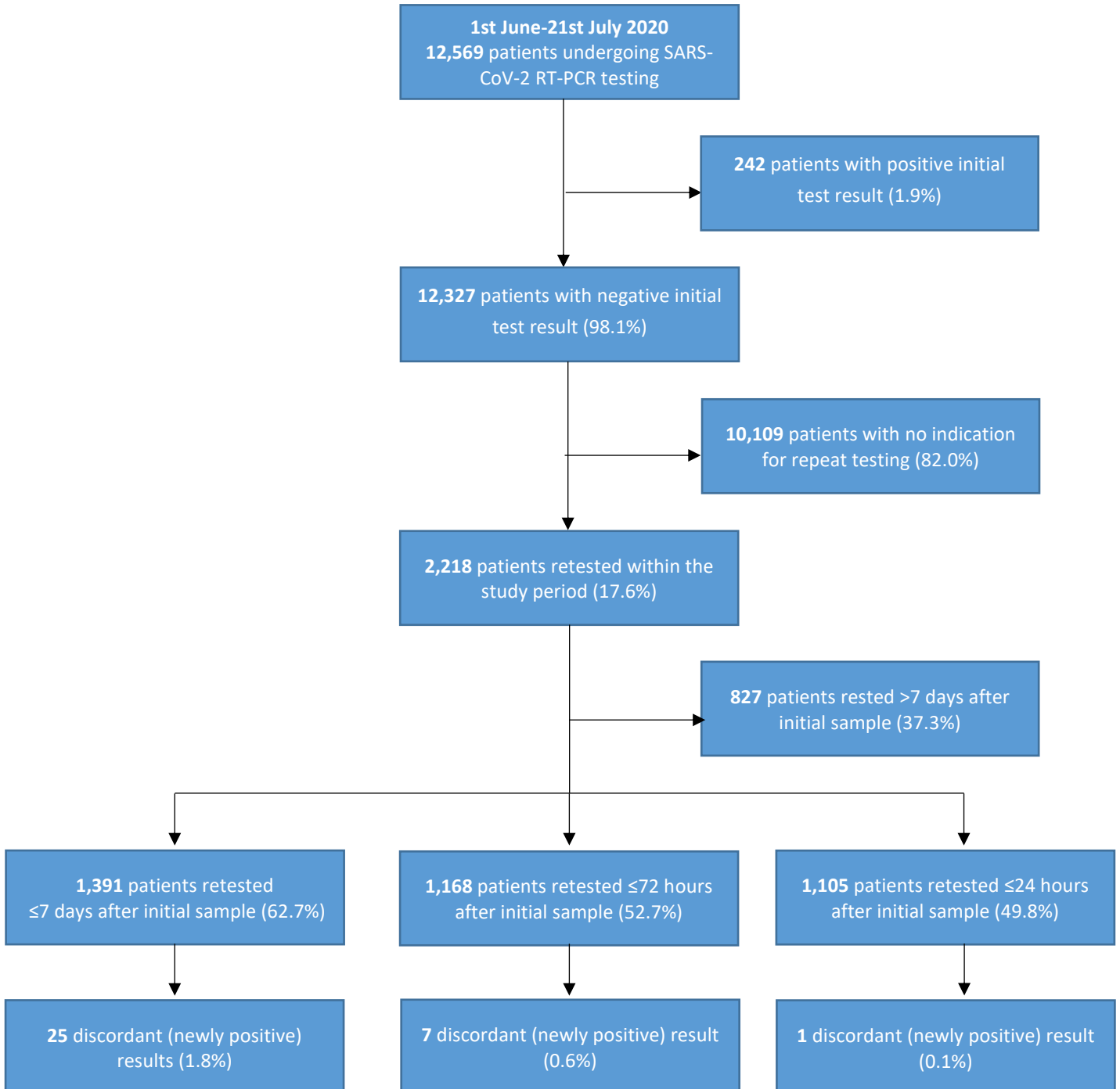
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Table 1. Characteristics of individuals tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by reverse-transcription polymerase chain reaction (RT-PCR) at the Royal Melbourne Hospital, Melbourne, Australia between 1st June and 21st July 2020.

Demographics	Total	Single test	Repeat test ≤7 days	Repeat test ≤72 hours	Repeat test ≤24 hours
Total individuals, n	12,569	10,318	1,391	1,168	1,105
Age, median [IQR]	35 [27-50]	34 [26-46]	61 [37-79]	67 [47-81]	69 [49-82]
Female, n (%)	7462 (59%)	6,228 (60%)	662 (48%)	523 (45%)	483 (44%)
SARS-CoV-2 positive, n (%)	286 (2.3%)	209 (2.0%)	25 (1.8%)	7 (0.6%)	1 (0.1%)
Negative to positive individuals					
Total individuals, n	-	-	25	7	1
Age, median [IQR]	-	-	31 [27-82]	37 [30-61]	39
Female, n (%)	-	-	18 (72%)	5 (71%)	1 (100%)
Interval negative to positive result, Median [IQR] (days)	-	-	3.3 [3.0-4.1]	2.6 [1.7-2.9]	0.9
Epidemiological Risk Factors for COVID-19					
Yes	-	-	25 (100%)	7 (100%)	1 (100%)
1 risk factor, n (%)	-	-	17 (68%)	5 (71%)	1 (100%)
2 risk factors, n (%)	-	-	8 (32%)	2 (29%)	-
COVID-19 contact	-	-	12 (48%)	3 (43%)	-
Confirmed outbreak	-	-	10 (40%)	4 (57%)	1 (100%)
Healthcare worker	-	-	11 (44%)	2 (29%)	-
Symptoms					
Initial swab					
Asymptomatic	-	-	12(48%)	3 (43%)	-
Symptomatic	-	-	12 (48%)	4 (57%)	1 (100%)
Unknown	-	-	1 (4%)	-	-
Repeat swab					
Asymptomatic	-	-	1 (4%)	-	-
Symptomatic	-	-	20 (80%)	6 (86%)	1 (100%)
Unknown	-	-	4 (16%)	1 (14%)	-

COVID-19, coronavirus disease 2019; IQR, inter-quartile range; n, number; SARS-CoV-2, severe acute respiratory syndrome coronavirus

Supplementary Figure 1. Flowchart of patients undergoing SARS-CoV-2 testing at the Royal Melbourne Hospital between 1st June and 21st July, 2020.



Supplementary Table 1. COVID-19 Screening and transmission-based precautions protocols

Suspected COVID-19 Criteria*	Number of negative SARS-CoV-2 RT-PCR results required prior to standing down transmission-based precautions#	
Fever OR chills in the absence of an alternative diagnosis that explains the clinical presentation OR Acute respiratory infection (eg. cough, sore throat, shortness of breath, runny nose, anosmia or loss of smell or loss of taste)	2 combined deep nasal and oropharyngeal swabs OR 1 combined deep nasal and oropharyngeal swab AND 1 lower respiratory tract sample	All patients requiring hospital admission that meet suspected COVID-19 criteria excluding those listed below
Testing note: Testing is also recommended for people with new onset of other clinical symptoms consistent with COVID-19 (including headache, myalgia, stuffy nose, nausea, vomiting, diarrhoea) AND who are close contacts of a confirmed case of COVID-19, who have returned from overseas in the past 14 days or who are healthcare or aged care workers.	1 combined deep nasal and oropharyngeal swab	1. All patients requiring hospital admission that meet suspected COVID-19 criteria AND Have an alternative explanation for their symptoms AND Do not have pneumonia AND Do not have epidemiological risk factors for COVID-19 OR 2. Ambulatory patients that meet suspected COVID-19 criteria OR 3. Asymptomatic patients prior to operative management or multiple aerosol generating procedures

*Victorian Department of Health and Human Services guidelines for suspected COVID-19¹.

#Royal Melbourne Hospital suspected COVID-19 screening guidelines.

Note: Limited asymptomatic COVID-19 screening of healthcare workers and close contacts of known COVID-19 cases was also undertaken during this period.

¹ Victorian Department of Health and Human Services. Coronavirus disease 2019 (COVID-19): case and contact management guidelines for health services and general practitioners. Version 23. Victorian Department of Health and Human Services, Melbourne 2020.