REPEAT TESTING

RECOMMENDATION

We suggest to repeat RT-PCR testing when the initial RT-PCR test is negative in symptomatic patients with high index of suspicion for COVID-19 infection. *(Low quality of evidence; Conditional recommendation)*

Consensus Issues

The recommendation applies only to symptomatic patients with high index of suspicion for COVID-19. Since the disease severity and the level of suspicion were not clearly defined in the studies, the level of suspicion may vary. Moreover, no specific recommendation was made regarding the specific time interval between the initial and the repeat test as well as the frequency of repeat PCR tests.

EVIDENCE SUMMARY

Should repeat RT-PCR testing after an initial negative RT-PCR versus single RT-PCR testing be done to diagnose COVID-19 in symptomatic patients with high index of suspicion?

Evidence Reviewers: Eva I. Bautista, MD, MSc; Aldrich Ivan Lois Burog, MD; Howell Henrian G. Bayona, MSc

Key Findings

Two cohort studies involving 368 patients were found on the accuracy of repeat RT-PCR testing after an initial negative test to diagnose COVID-19 in symptomatic patients with high index of suspicion. The evidence was assigned a low quality rating due to serious risk of bias and serious imprecision. The sensitivity of repeat RT-PCR testing ranged from 0.83 (95% CI 0.75-0.90) to 0.85 (95% CI 0.62-0.97) or about 15% higher compared to the sensitivity of a single RT-PCR test, which ranged from 0.68 (95% CI 0.58-0.76) to 0.70 (95% CI 0.46-0.88). Specificity of repeat testing was consistently very high (1.00, 95% CI 0.89-1.00).

Introduction

False negative rates for single RT-PCR have been reported to occur in 7% (5 in 70) [1] to 19% [2-4] in suspected COVID-19 patients. These are higher than the acceptable false negative rate of <2%. [5] RT-PCR sensitivity is affected by duration of illness (i.e., time between symptom onset to testing), specimen location (URT vs LRT) [6-8] and cycle threshold for positivity [9]. Repeat testing is usually done 24 hours to 4 days after a negative initial RT-PCR among hospitalized adults suspected to have COVID-19.

High index of suspicion for COVID-19 infection is considered in symptomatic patients presenting with, but not limited to:

a. Clinical deterioration in the presence of an established disease etiology and with adequate treatment and severe or progressive disease, with possible coinfection with COVID-19.
b. No other etiology for the patient's signs and symptoms has been identified despite work-up.
c. Clinical specimen(s) initially sent was/were deemed to be unsatisfactory or insufficient (delay in transport and processing, only NPS or OPS was sent) [10].

Review Methods

Search was conducted in databases such as MEDLINE and Cochrane. Preprints were also searched in MedRxiv, ChinaXiv, and BioRxiv databases. Search was conducted using the following search terms: COVID-19, SARS-CoV-2, NAAT, RT-PCR, PCR, repeat testing, repeat test, cumulative test and serial test. . Reference lists of selected articles were reviewed for inclusion. Two reviewers independently screened titles and abstracts initially, then the eligible full-text studies.

We included cohort, cross-sectional and case-control studies that evaluated diagnostic test accuracy and provided complete data to estimate both the sensitivity and specificity of the index test. We excluded studies that used a combination of tests and clinical criteria as reference standard. Studies with inconclusive results for patients were excluded from analysis. We also excluded studies that reported on the number of specimens, rather than number of patients.

Results

Characteristics of included studies

Two pre-print cohort studies involving 366 hospitalized adult patients and 2 children with suspected COVID-19 of varying severity and had initial RT-PCR testing [11,12]. One study involved 53 patients who underwent a second RT-PCR test after 3 days and compared against metagenomic sequencing as reference standard [12]. The other study enrolled 315 patients and employed repeat/cumulative RT-PCR testing every 24 hours for up to at most 5 tests [11].

Methodological quality

Overall quality of evidence was rated **low** due to serious risk of bias and serious imprecision. Type of specimen (throat swab vs nasopharyngeal swab), differences in timing of specimen collection from symptom-onset and reference test not interpreted independently from index test were the sources of bias, while imprecision was attributed to small sample size in the studies.

Diagnostic accuracy

The sensitivity of repeat RT-PCR testing ranged between 0.83 (95% CI 0.75-0.90) to 0.85 (95% CI 0.62-0.97), which is about 15% higher compared to the sensitivity of a single RT-PCR test, which ranged from 0.68 (95% CI 0.58-0.76) -0.70 (95% CI 0.46-0.88). Specificity of repeat testing was consistently very high at 1.00 (95% CI 0.89-1.00).

Benefits and harms

Both studies did not specifically report on patient outcomes nor harm. Based on the resulting diagnostic performance measures, it can be derived that repeat RT-PCR testing reduces false negative rate of single RT-PCR by about 15% from 30% to 15% in one study and from 32% to 17% in another. This is still a high false negative rate compared to the acceptable false negative rate of 2% determined by IDSA [5].

Recommendations from other groups

CDC (07 January 2021) recommends repeat testing of symptomatic individuals in certain healthcare settings (e.g., patients at risk for severe illness, critical nature of healthcare personnel, challenges with social distancing, assisted living facilities, intermediate care facilities for individuals with intellectual disabilities, institutions for mental disease, and psychiatric residential treatment facilities) [13]

IDSA (23 December 2020) and PSMID (29 July 2020) recommend repeat testing for symptomatic patients with an initial negative COVID-19 test result if there is intermediate [5] to high index of suspicion for COVID-19 infection. [10] Such conditions include, but are not limited, to the following:

a. Clinical deterioration in the presence of an established disease etiology and with adequate treatment. A single negative test result, particularly if this is from an upper respiratory tract specimen, does not exclude infection. Repeat sampling and testing, preferably of lower respiratory specimen, is strongly recommended in severe or progressive disease. Consider a possible coinfection with COVID-19.
b. No other etiology for the patient's signs and symptoms has been identified despite work-up.
c. Clinical specimen(s) initially sent was/were deemed to be unsatisfactory or insufficient (delay in transport and processing, only NPS or OPS was sent).
d. Repeat testing is recommended 24-48 hours after an initial negative test. [5]

Research gaps

There were no ongoing or planned studies found related to this topic.

References

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Appendix 1: Characteristic of Included Studies

Author	Study Design	Sample Size	Population	Index Test/s	Reference Test	Outcome
Ai J (Apr 2020)[10]	Cohort	315	315 adults hospitalized, suspected COVID-19	Two to five repeat RT-PCR done at 24 hours' interval	Positive in any 1 to at most 5 repeat/ cumulative RT-PCR testing	Sensitivity Specificity
Ai JW (Feb 2020)[11]	Multi-center Cohort	53	53 Suspected COVID-19	Repeat RT-PCR three days after initial RT-PCR test	metagenomic sequencing	Sensitivity Specificity

Appendix 2: GRADE Evidence Profile

Question: Should [Repeat RT-PCR] after an initial negative test vs. [Single RT-PCR] be used to diagnose [COVID-19] in [suspected COVID-19 patients]?

[Repeat RT-PCR] after an initial negative test		[Single RT-PCR]	
Sensitivity	0.83 to 0.85	Sensitivity	0.68 to 0.70
Specificity	0.89 to 1.00	Specificity	0.89 to 1.00

Prevalences	10%	40%	0%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested						Test accuracy CoE
								pre-test probability of 10%		pre-test probability of 40%		pre-test probability of 0%		
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	
True positives (patients with [COVID-19])	2 studies 128 patients	cross-sectional (cohort type accuracy study)	serious [1,a]	not serious	not serious	serious [2,b]	none	83 to 85	68 to 70	332 to 340	272 to 280	0 to 0	0 to 0	⨁⨁◯◯ LOW
								15 more to 15 more TP in [Repeat RT-PCR] after an initial negative test		**60 more to 60 more TP in [Repeat RT-PCR] after an initial negative test**		**0 fewer to 0 fewer TP in [Repeat RT-PCR] after an initial negative test**		
False negatives (patients incorrectly classified as not having [COVID-19])								15 to 17	30 to 32	60 to 68	120 to 128	0 to 0	0 to 0	
								15 fewer to 15 fewer FN in [Repeat RT-PCR] after an initial negative test		**60 fewer to 60 fewer FN in [Repeat RT-PCR] after an initial negative test**		**0 fewer to 0 fewer FN in [Repeat RT-PCR] after an initial negative test**		
True negatives (patients without [COVID-19])	2 studies 240 patients	cross-sectional (cohort type accuracy study)						801 to 900	801 to 900	534 to 600	534 to 600	890 to 1000	890 to 1000	-
								0 fewer to 0 fewer TN in [Repeat RT-PCR] after an		**0 fewer to 0 fewer TN in [Repeat RT-PCR] after an**		**0 fewer to 0 fewer TN in [Repeat RT-PCR] after an**		

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested						Test accuracy CoE
								pre-test probability of 10%		pre-test probability of 40%		pre-test probability of 0%		
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	[Repeat RT-PCR] after an initial negative test	[Single RT-PCR]	
								initial negative test		**initial negative test**		**initial negative test**		
False positives (patients incorrectly classified as having [COVID-19])								0 to 99	0 to 99	0 to 66	0 to 66	0 to 110	0 to 110	
								0 fewer to 0 fewer FP in [Repeat RT-PCR] after an initial negative test		**0 fewer to 0 fewer FP in [Repeat RT-PCR] after an initial negative test**		**0 fewer to 0 fewer FP in [Repeat RT-PCR] after an initial negative test**		

Explanations

a. There was no independent interpretation of the reference test from the index test.
b. The sample size was small.

References

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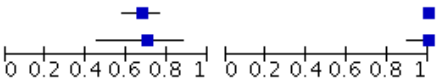
Appendix 3: Forest plot showing sensitivity and specificity of single and repeat RT-PCR testing

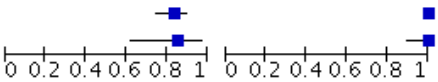
Single RT-PCR

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Ai J Apr 2020	73	0	35	207	0.68 [0.58, 0.76]	1.00 [0.98, 1.00]		
Ai J Feb 2020	14	0	6	33	0.70 [0.46, 0.88]	1.00 [0.89, 1.00]		



Cumulative RT-PCR

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Ai J Apr 2020	90	0	18	207	0.83 [0.75, 0.90]	1.00 [0.98, 1.00]		
Ai J Feb 2020	17	0	3	33	0.85 [0.62, 0.97]	1.00 [0.89, 1.00]		



Appendix 4: Risk of bias and applicability concerns summary