### Key Findings

- The overall ‘clinical’ false negative rate (1 – sensitivity) of rt-PCR in the detection of SAR-CoV-2 in the respiratory tract is estimated to be 30%, although estimates vary from 3.5 – 50%. This rate is higher when the technique of clinical sampling is poor, higher in nasopharyngeal and oral swabs than lower respiratory tract secretions, and higher early in the course of disease (prior to symptom development) and following the first week of symptoms.

- The performance of the rt-PCR test can be maximized by:
  1. Timing and collecting specimens in the optimal manner
  2. Applying the test in those individuals with a high pre-test probability of having COVID-19; this includes individuals living in communities with a high prevalence of disease, those with an epidemiological history linking them to a confirmed case, and those with symptoms associated with

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**Rapid Review Report**

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<th><strong>Review Title:</strong></th>
<th>What factors can be used to identify negative PCR tests that are ‘false negatives’?</th>
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<td>rt-PCR false negatives</td>
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<td><strong>Date/Time:</strong></td>
<td>May 25, 2020</td>
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<td><strong>Version:</strong></td>
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<td><strong>Revision History:</strong></td>
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<td><strong>Prepared By:</strong></td>
<td>Andreea Badea, Community Health and Epidemiology, University of Saskatchewan</td>
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<td>Michelle Dalidowicz, Clinical Librarian, Saskatchewan Health Authority Library</td>
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<td>Lukas Miller, Clinical Librarian, Saskatchewan Health Authority Library</td>
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<td><strong>Peer Reviewer:</strong></td>
<td>Bruce Reeder, College of Medicine, University of Saskatchewan</td>
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<td><strong>Contact:</strong></td>
<td>For questions specific to this review, contact <a href="mailto:bruce.reeder@usask.ca">bruce.reeder@usask.ca</a></td>
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<td><strong>Cite As:</strong></td>
<td>Badea, A; Reeder, B; Young, C; Dalidowicz, M; Miller, L. What factors can be used to identify negative PCR tests that are ‘false negatives’? 2020 May 25; Document no.: EOC052101 RR. In: COVID-19 Rapid Evidence Reviews [Internet]. SK:  SK COVID Evidence Support Team, c2020. 12 p. (CEST rapid review report)</td>
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</table>
COVID-19, potentially identified with the aid of a clinical prediction rule
3. Serial testing with a repeat rt-PCR test after a specific interval such as 24-48 hours
4. In the clinical setting, the additional assessment of blood biomarkers, IgM/IgG serology and chest CT scan
• These considerations apply to the decision to conduct initial rt-PCR testing as well as to the decision to re-test individuals with an initial negative result.

Limitations
• A number of the references are available only as preprints and are pending peer review

GRADE of Evidence: B - Moderate
A grade of "B" is assigned when further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate. The review may include one high quality study and/or several studies with some limitations.

For more information about how this rating was determined, visit https://www.essentialevidenceplus.com/product/ebm_loe.cfm?show=grade

Background/Context
Since the beginning of the pandemic, diagnostic testing of symptomatic individuals for the presence of the novel coronavirus SARS CoV-2 in throat, nasopharyngeal or respiratory secretions using real time Polymerase Chain Reaction (rt-PCR) has been the standard of care. Now, with enhanced laboratory capacity, consideration is being given to testing: all patients upon admission to hospital for a stay longer than 24 hours; immunocompromised asymptomatic individuals; health staff working with immunocompromised patients; anyone working outside the home; individuals at their workplaces; and vulnerable populations.

The rt-PCR test has a high ‘analytical’ sensitivity and specificity (99%, 100%, respectively) [Lu], that is, when the test is performed on known positive and negative samples. However, its ‘clinical’ sensitivity is considerably less, varying according to the type of sample collected (nasopharyngeal, oral, lower respiratory secretions), the technical adequacy of specimen collection, specimen preservation and transport, and the timing of sample collection. Hence, the false negative rate (1 – sensitivity) may be vary considerably.

As rt-PCR testing is expanded in the province, a rise in the number of false negative tests can be expected.

Purpose
To better identify false negative rt-PCR results and delineate maneuvers to reduce the number of false negative tests.

Review Question(s)
• What factors can be used to identify negative PCR tests that are ‘false negatives’?
Method
This Rapid Review was produced within 48 hours of request.

Summary of Evidence
A rapid review conducted by the British Columbia Centers for Disease Control [BCCDC] concludes that rt-PCR testing on a single occasion has an clinical sensitivity of approximately 70% (false negative rate 30%), with estimates ranging from 90% in small Canadian studies to as low as 50% in some international studies. A positive rt-PCR test indicates COVID-19 infection with a high degree of certainty, since the test has a high specificity and positive predictive value, however a negative test can be falsely negative due to improper specimen collection and storage, the anatomical origin of the specimen (upper vs. lower respiratory tract) and the timing of the test in the course of the illness. Detectable viral loads are higher in lower respiratory tract secretions than upper [Yang; Wang] and increase progressively from the time of exposure to the development of clinical symptoms, then generally begin to decrease 7-10 days following symptom development [To; He].

In view of these considerations, it is not surprising that several studies demonstrate an increased detection of cases when repeat rt-PCR testing is performed on individuals with an initial negative result [Green; Long D; Zhang] Two large retrospective analyses of laboratory results in the US are instructive. The New York-Presbyterian laboratories performed rt-PCR tests on 22,338 patients [Green]. Repeat tests were performed over a median of 8 days (range 1-49) on a non-random subset of 2,413 patients who had an initial negative result; 18.6% of this group became positive on repeat testing in a generally linear fashion out to 40 days from initial testing. Repeat testing increased the clinical sensitivity of rt-PCR testing in this setting from 58.1% to 66.2%. In the second laboratory study from Stanford University and the University of Washington of 20,912 patients tested, 626 were retested within 7 days [Long D]. Only 3.5% of these individuals had a positive result on retesting. In each study the group that was retested is unlikely to be representative of the total population tested, since retesting was likely influenced by the clinical presentation of the patients, and in these studies it is unclear whether hospitalized and non-hospitalized patients or both were included.

A systematic review [Arevalo-Rodriguez] which examined the results of five studies that had enrolled 957 patients derived a pooled estimation of false negative rate of 8.5% (range in the studies: 2-29%). However, the authors assessed the quality of the reviewed studies to be low.

A high-quality review of 7 studies examining 1330 respiratory samples permitted Kucirka and colleagues to model the changes to the sensitivity, and so false negative rate (1-sensitivity), of rt-PCR testing of nasopharyngeal/oral secretions over time since exposure [Kucirka]. The authors estimate that the false negative rate of the test decreases from 100% on the day 1, to 67% on day 4, 38% on day 5 (median day of onset of symptoms), 20% on day 8 (day 3 of symptoms), then increases progressively to 66% on day 21. They go on to demonstrate the relative utility of diagnostic rt-PCR testing over time in a patient population with a pre-test probability (prevalence) of 11.2% as was seen in a large study of household contacts in Shenzhen, China [Bi]. The accompanying figure from their paper reflects the changes in the false negative rate over time and demonstrates that rt-PCR is a more useful diagnostic tool when the pre-test probability is high.
To minimize false negative results of rt-PCR testing in all settings, effort must be made to optimize the technique of sample collection, preservation, and transport. In clinical settings, the test should be performed when its sensitivity is maximized, that is, from the time of onset of symptoms for a period of 7-10 days. When used as a screening maneuver in an asymptomatic population or when repeat rt-PCR is being considered following an initial negative result, it will be useful to identify individuals with a higher pre-test probability in whom the test will perform better, that is, be more discriminating. To identify such individuals, one needs to consider the disease prevalence in the community in which they live, their epidemiological history of contact with confirmed COVID-19 cases, and the presence or absence of clinical symptoms. An early systematic review found that available disease prediction models were poorly reported, likely biased, and overly optimistic [Wynants]. A more recent report based on well-conducted study of 803 healthcare workers from the Netherlands is somewhat more promising [Tostmann]. It proposes a weighted symptom checklist: 3 for anosmia, 2 for muscle ache, 1 each for extreme tiredness, headache, ocular pain, fever, and general malaise, with a score of 3 or greater representing a positive screen. With this tool, the authors report a sensitivity of 91.2% (false negative rate = 8.8%) and specificity of 55.6% for the identification of rt-PCR positive individuals.

Further maneuvers to identify false negative rt-PCR results when suspicion is high may include serial testing with a repeat rt-PCR test within 24-48 hours [Green], the measurement of blood biomarkers

Figure 3. Posttest probability of SARS-CoV-2 infection after a negative RT-PCR result, by pretest probability of infection.

RT-PCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
There is some evidence that digital PCR testing may be more sensitive than standard rt-PCR testing in the detection of SARS-CoV-2 [Lu; Suo].

**Conclusions**

The overall clinical false negative rate (1 – sensitivity) of rt-PCR in the detection of SAR-CoV-2 in the respiratory tract is estimated to be 30%, although estimates vary from 3.5 – 50%. This rate is higher when the technique of clinical sampling is poor, higher in nasopharyngeal and oral swabs than lower respiratory tract secretions, and higher early in the course of disease (prior to symptom development) and following the first week of symptoms. Performance of the rt-PCR test can be optimized by timing and collecting specimens in an optimal manner and by applying the test in those individuals with a high pre-test probability of having COVID-19. This includes individuals living in communities with a high prevalence of disease, those with an epidemiological history linking them to a confirmed case, and those with symptoms associated with COVID-19 identified potentially with the aid of a clinical prediction rule. Approaches to reduce the false negative rate include rt-PCR re-testing after a specific period of time such as 24-48 hours, measurement of blood biomarkers, IgM/IgG serology, and chest CT scan. These considerations apply to initial rt-PCR testing as well as to the decision to re-test an individual with a negative result.

**Glossary**

(Optional, but useful if there are clinical/statistical terms being referenced in the document.)
### Table 1: Summary of Literature

<table>
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<tr>
<th>Ref</th>
<th>Sample/population</th>
<th>Method</th>
<th>Primary outcome measure</th>
<th>Additional findings</th>
<th>Quality of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ai</td>
<td>315 hospitalized patients, 108 COVID, 207 non-COVID</td>
<td>Area under curve of 1&lt;sup&gt;st&lt;/sup&gt; RT-PCR: 0.84 Cumulative 2&lt;sup&gt;nd&lt;/sup&gt; 0.92 Cumulative 3&lt;sup&gt;rd&lt;/sup&gt; 0.96</td>
<td>Age threshold to predict COVID 41.5y (sensitivity 0.70) Relatively high false negative – if first test negative but clinically symptomatic, reassess</td>
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<tr>
<td>2. Arevalo</td>
<td>957 patients</td>
<td>Systematic review</td>
<td>Pooled estimation of false negative proportion 0.085, highly affected by unexplained heterogeneity</td>
<td>High risk of bias, indirectness and inconsistency, systematic review found up to 29% false negatives</td>
<td>Certainty very low</td>
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<td>3. Artesi</td>
<td></td>
<td></td>
<td>Mutation of virus could affect accuracy of RT-PCR</td>
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<td>13. Ferrari</td>
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<td></td>
<td>15-20% false negatives of RT-PCR as common knowledge stat</td>
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<td>14. Green</td>
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<td>Sensitivity of single test 66.2%, 95.6% of repeated testing, depending on the unknown number of false negatives in single tested</td>
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<tr>
<td>15. Guo</td>
<td>208 plasma samples from 82 confirmed cases and 58 probable cases (negative RT-PCR but typical clinical manifestation)</td>
<td>Positive detection of single qPCR 51.9%</td>
<td>but when combined with IgM ELISA rose to 98.6%</td>
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<td>17. He</td>
<td>82 hospitalised patients – 34 COVID and 48 non-COVID by RT-PCR</td>
<td>RT-PCR sensitivity 79% in COVID patients</td>
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<td>21. Kinlock</td>
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<td>Improper collection can lead to higher rates of false negatives</td>
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<td>24. Kucirka</td>
<td>633 nasal swabs from exposed individuals</td>
<td>100% false negative on day 1 (day of exposure), 61% by day 4. Day 5 (symptom onset) 39% and day 8 26% after which it begins to rise again</td>
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<td>Authors</td>
<td>Description</td>
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<td>25.</td>
<td>Kucirka</td>
<td>1,330 nasal swabs of exposed individuals</td>
<td>100% false negative day 1 (day of exposure), 67% day 4, 38% day 5 (symptom onset), 20% day 8 after which it begins to rise again</td>
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<td>30.</td>
<td>Li</td>
<td>610 hospitalized patients, clinically diagnosed with COVID-19</td>
<td>63% negative on 1st RT-PCR, of those, 72.9% negative on 2nd test</td>
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<td>Several tests at different time points were variable from the same patients</td>
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<td>33.</td>
<td>Liu</td>
<td>133 hospitalized patients</td>
<td>65.9 – 71.15% positive ratio of RT-PCR depending on severity of disease</td>
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<td>34.</td>
<td>Liu</td>
<td></td>
<td>Non-specific primers can lead to higher rates of false negatives</td>
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<td>35.</td>
<td>Long</td>
<td>36 hospitalized COVID patients</td>
<td>83% positive on 1st RT-PCR, of the remaining 6, 3 positive on 2nd RT-PCR and 3 only positive on 3rd RT-PCR</td>
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<td>36.</td>
<td>Long</td>
<td>23,126 RT-PCR on 20,912 patients at University of Washington and Stanford</td>
<td>626 who tested negative on 1st RT-PCR were re-tested and only 3.5% became positive on 2nd test within 7 days, rest continued to test negative on all subsequent tests</td>
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<td>Very low false negative rate</td>
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<td>37.</td>
<td>Lu</td>
<td>108 specimens from 36 hospitalized patients</td>
<td>Digital PCR accuracy 96.3%</td>
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<td>46.</td>
<td>Padhye</td>
<td>1014 hospitalised patients in Wuhan, of which 601 were COVID patients</td>
<td>RT-PCR sensitivity 0.77.</td>
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<td>For a patient with a prior probability of COVID of greater that 18%, at least 2 negative RT-PCR tests are needed to lower the chance of COVID to less than 5%</td>
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<td>58.</td>
<td>Suo</td>
<td>57 patients tested negative by RT-PCR – 43 in clinic with fever and 14 supposed convalescents about to be discharged post-tx</td>
<td>33/35 negative RT-PCR corroborated by digital droplet PCR, but 64% of convalescent patients with negative RT-PCR tested positive by ddPCR</td>
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<td>64.</td>
<td>Vogels</td>
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<td>Different brands of RT-PCR tests have different abilities</td>
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<td>to differentiate between true negatives and low levels of virus</td>
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References Included in Summary


BCCDC. Interpreting the results of nucleic acid amplification testing (NAT; or PCR tests) for COVID-19 in the respiratory tract. Available at: http://www.bccdc.ca/Health-Professionals-Site/Documents/COVID19_InterpretingTesting_Results_NAT_PCR.pdf


Long D, Gombar S, Hogan C et al. Occurrence and Timing of Subsequent SARS-CoV-2 RT-PCR Positivity Among Initially Negative Patients. https://doi.org/10.1101/2020.05.03.20089151doi


Yang Yang, Minghui Yang, Chenguang Shen, Fuxiang Wang, Jing Yuan, Jinxiu Li, Mingxia Zhang, Zhaoqin Wang, Li Xing, Jinli Wei, Ling Peng, Gary Wong, Haixia Zheng, Mingfeng Liao, Kai Feng, Jianming Li, Qianting Yang, Juanjuan Zhao, Zheng Zhang, Lei Liu, Yingxia Liu. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv 2020.02.11.20021493; https://doi.org/10.1101/2020.02.11.20021493


Appendix: Evidence Search Details

Search Strategies

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S12 7,471
S11 (MH "False Negative Results") OR TI (false negative* or true negative*) OR AB (false negative* or true negative*) 7,471
S10 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 Limiters - Published Date: 20191201-20201231; English Language 3,491
S9 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 15,397
S8 TX ("severe acute respiratory syndrome"* or Huanan*) 3,735
S7 TX ((outbreak* or wildlife* or pandemic* or epidemic*) N1 (Wuhan* or Hubei or China* or Chinese* or Huanan*)) 684
S6 TX ("seafood market"* or "food market"* or pneumonia*) N10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)) 467
S5 TX (respiratory* N2 (symptom* or disease* or illness* or condition*) N10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)) 1,254
S4 TX ("2019-nCoV" or 2019nCoV or nCoV2019 or "nCoV-2019" or "COVID-19" or COVID19 or "CORVID-19" or CORVID19 or "WN-CoV" or WNCov or "HCoV-19" or HCoV19 or "2019 novel*" or Ncov or "n-cov" or "SARS-CoV-2" or "SARSCoV-2" or "SARS-CoV2" or "SARS-CoV2" or SARSCov19 or "SARS-Cov19" or "SARSCoV-19" or "SARS-Cov-19" or Ncovor or Ncorona* or Ncorono* or NcovWuhan* or
NcovHubei* or NcovChina* or NcovChinese* or SARS2 or "SARS-2" or SARScoronavirus2 or "SARS-coronavirus-2" or "SARScoronavirus 2" or "SARS coronavirus2" or SARScoronovirus2 or "SARS-coronovirus-2" or "SARScoronovirus 2" or "SARS coronavirus2") 3,103
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7 ("seafood market*" or "food market*" or pneumonia*) adj10 (Wuhan* or Hubei* or China* or Chinese* or Huanan*).ti,ab,kw. (1373)
8 ((outbreak* or wildlife* or pandemic* or epidemic*) adj1 (Wuhan* or Hubei* or China* or Chinese* or Huanan*)).ti,ab,kw. (108)
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10 or/1-9 (38850)
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18 15 or 17 (34)

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NcovChina* or NcovChinese* or SARS2 or "SARS-2" or SARScoronavirus2 or "SARS-coronavirus-2" or SARScoronavirus 2" or "SARS coronavirus2" or SARScoronovirus2 or "SARS-coronovirus-2" or SARScoronovirus 2" or "SARS coronovirus2").ti,ab,kw,kf. (14538)
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9 or/1-8 (37068)
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15 ("polymerase chain reaction" or PCR or qPCR or rPCR or RT-PCR) adj5 (false negative* or true negative*).af. (965)
16 11 and 15 (11)
17 14 or 16 (34)

Sources
- (Particular databases, was grey literature included, etc.)
- Refer to the evidence search report for extensive sources. Be sure to include any additional resources not referenced in the evidence search report.
- This field is mandatory.

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