Woloshin S, Patel N, Kesselheim AS. **False Negative Tests for SARS-CoV-2 Infection - Challenges and Implications** [published online ahead of print, 2020 Jun 5]. *N Engl J Med*. 2020;10.1056/NEJMp2015897. doi:10.1056/NEJMp2015897

This article examines the impact of RT-PCR test sensitivity and prior probability of SARS-CoV-2 infection on the probability of infection given a negative test result and considers the implications of false negatives tests for SARS-CoV-2.

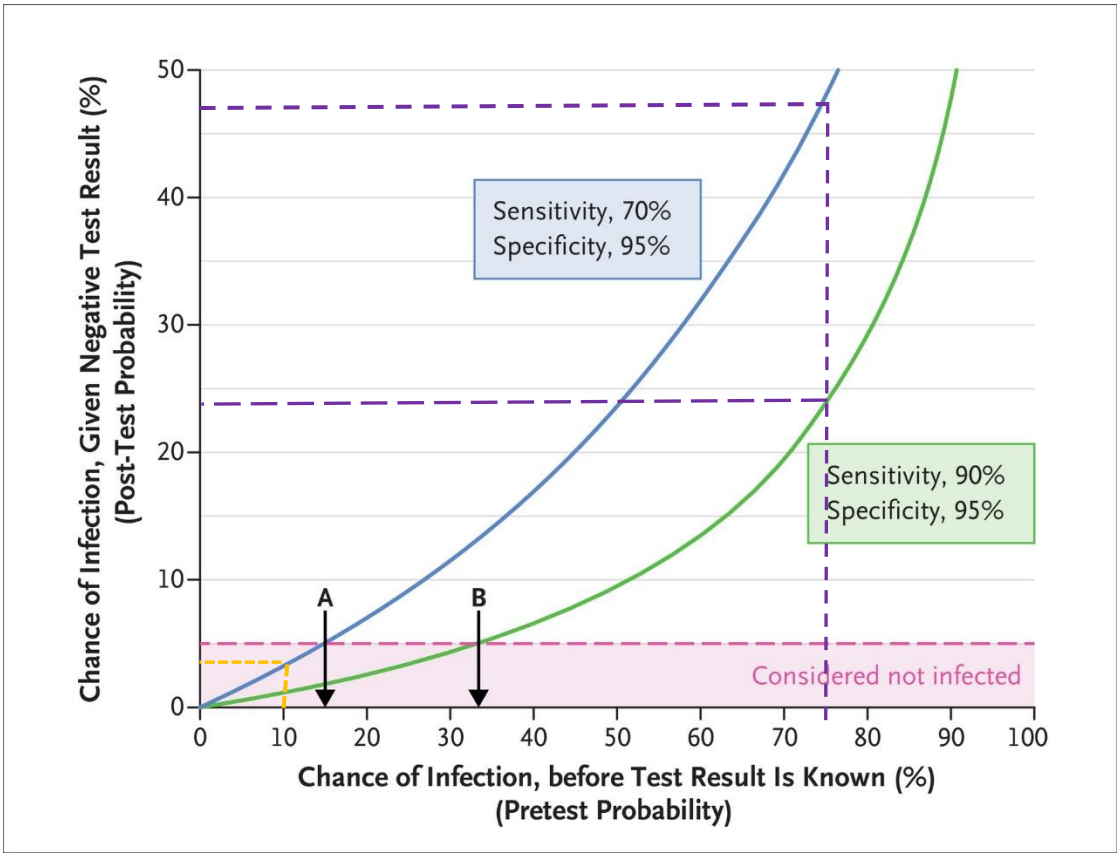
METHODS The article refers to three studies reporting on the sensitivity of RT-PCR tests for detecting viral RNA in people with apparent Covid-19 illness. These studies (comprising a systematic review not peer reviewed, and two diagnostic accuracy studies one of which has not been peer reviewed), suggest that sensitivity of the RT-PCR test may be as low as 70% (though the certainty of this evidence is limited by methodological flaws in the studies). Using a pre-test post-test probability plot (covering only the post test probability from 0-50% and for the situation where a test is negative), the article shows the post-test probability of infection at a test sensitivity of 70% and 95%. The article uses a threshold of 5%, below which it would be reasonable to act as if the person did not have the infection (pink area on the plot below).

RESULTS The article demonstrates the need for highly sensitive tests for SARS-CoV-2. From the plot it can be seen that with a negative test result for a test with 70% sensitivity (blue line on the plot), the threshold is exceeded when the pre-test probability of infection reaches 15% (point A on the plot). When the higher sensitivity test is used (green line on the plot; sensitivity 95%), the post-test probability of infection would remain below 5% even if the pre-test probability were as high as 33%. The plot illustrates that at high pre-test probability values, testing is of little value because negative test results are not able to lower the probability of infection below the proposed threshold.

DISCUSSION For this article, discussion focused around the properties of diagnostic tests, the determination of test thresholds and pretest probability of infection.

- The properties of diagnostic test accuracy; sensitivity and specificity were revised. Sensitivity being a measure of how sensitive the test is to the presence of disease. A test with 100% sensitivity detects everyone with disease (the test does not miss cases). Specificity is a measure of how many people without disease are identified as not having the disease. When test sensitivity and specificity are below 100%, false negative (a test result is negative when the individual truly has disease) and false positive errors (a test result is positive when the individual does not have the disease) result. Bayes theorem, which incorporates information about test accuracy and an individual's pretest probability (an estimate of the chance of being infected before testing) can be used to determine the probability of disease after testing.
- The article used a threshold of 5% which is the probability below which an individual would be considered not infected. It is the probability of a person being a false negative – the individual has the infection but is treated as though they are not infected. Thresholds are necessary because tests are imperfect (though these thresholds are often implicit). Setting of thresholds is a value judgment and will vary according to the context in which the test will be used. For example, the threshold may be lowered for an indigenous community where mortality rate from SARS-CoV-2 would be expected to be higher (at the lower threshold there is less chance of missing cases but the tradeoff is more people will need to be quarantined).
- Estimating the pretest probability of disease is challenging. Using pretest probabilities of infection among contacts of a person with SARS-CoV-2 in Germany (Bohmer et al 2020), the impact of test sensitivity and pretest probability can be demonstrated. Given a pretest probability of infection of 10% in a household contact of an individual before they are detected as being infected, the post-test probability of infection with a negative test in that contact is below the threshold for both tests (orange dotted lines). For an individual in shared isolation with a case and a pre-test probability of infection of 75%, tests of 70 and 95% sensitivity will not reduce the individual to below the threshold (purple dotted lines).
- Usually sensitivity and specificity are assumed to be fixed quantities of a test. Evidence does suggest however, that RT-PCR test sensitivity may vary over time.

OVERALL SUMMARY By demonstrating the limited value of tests when pre-test probability of infection is high – the article highlights the importance of strategies to reduce pre-test probability of infection (e.g. by social distancing). Even a highly sensitive test cannot rule out infection when an individual with a high pre-test probability returns a negative test. The article suggests therefore that an individual with a high pre-test probability (e.g. displaying typical symptoms and has a known exposure) returning a negative test should be assumed to be a false negative. The article emphasises that information on test sensitivity in asymptomatic people is currently unknown, but urgently needed and that RT-PCR tests for SARS-CoV-2 should be rigorously evaluated in real life situations and test sensitivity and specificity provided at the time of market authorisation.


Chance of Infection, Given Negative Test Result (%)
(Post-Test Probability)
50
40
30
20
10
0
Sensitivity, 70%
Specificity, 95%
Sensitivity, 90%
Specificity, 95%
A
B
Considered not infected
0
10
20
30
40
50
60
70
80
90
100
Chance of Infection, before Test Result Is Known (%)
(Pretest Probability)