PRELIMINARY REPORT

Title: **Positive and Negative Predictive Values and their Impact on RT-PCR Diagnostic Testing for SARS-CoV-2**

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Abstract: Positive and negative predictive values (PPVs and NPVs) help researchers assess how reliable a medical test is. PPVs and NPVs for the RT-PCR test used for SARS-CoV-2 were estimated based on a range of published values for sensitivity, specificity and prevalence calculated from reported numbers of cases and tests. High, typical, and low values of sensitivity and specificity were applied to the world, Australia, the state of Victoria, and to the Pfizer and AstraZeneca mRNA vaccine trials, and the corresponding PPVs and NPVs were calculated for each situation. Due to the low prevalence of the disease in these populations, the resulting PPV values varied from 0 to 38.1%, while the NPVs remained above 99%. These results indicate the lack of practical value of the test results and the unreliability of using the RT-PCR test as a mass screening test, rather than a diagnostic aid for pre-screened patients with acute symptoms.

1. Introduction

Positive and negative predictive values (PPVs and NPVs) indicate the likelihood that a person does or does not have a condition being tested for, based on the results of the test. No test is 100% accurate, so the PPV and NPV can be used to give the diagnosing physician an indication of how trustworthy the results of a test are. The PPV gives the probability that a positive test result is a genuine indication of the underlying condition under test, whereas the NPV gives the probability that a negative test result reflects a genuine absence of the condition.

PPV and NPV depend on the prevalence of the condition in the population, the sensitivity of the test and the specificity of the test.

- Prevalence refers to the number of cases of a disease that are present in a particular population at a given time as a percentage of the population.
- Sensitivity refers to the ability of a test to detect the condition under test, and
- Specificity refers to its ability to discriminate between the condition under test and other conditions.

A test with a low sensitivity will miss a significant number of actual cases resulting in the generating false negative results, whereas a test which has a low specificity will give positive test results in patients who do not have the condition under test, generating false positive results.

PPVs and NPVs are often illustrated in a two-way table as shown below. In this example it is assumed that the prevalence of the condition is 0.2%, the sensitivity of the test is 90%, and the specificity of the test is 95%. It is assumed 1,000 people are tested.

	condition	not condition	tot
+ve	18	49	67
-ve	2	931	933
tot	20	980	1000

Since the prevalence of the condition is 0.2%, the total number expected to have the condition is 0.2% of 1,000 people or 20 people. With a sensitivity of 90% we would expect 18 out of 20 people with the condition to test positive, and two to test negative, generating false negatives.

With a specificity of 95% it would be expected that of the 980 people without the condition, 95% of 980, or 931 people would test negative, whereas 5% or 49 people would test positive, generating false positives.

The positive and negative predictive values are calculated below:

PPV = 18/(18+49) x 100 = 26.9%
NPV = 931/(2+931) x 100 = 99.8%

This means a person who tests positive has a 26.9% chance of actually having the condition under test, whereas a person who tests negative has a 99.8% chance of not actually having the condition. This example shows how conditions with a low prevalence in the population can have a low PPV even when the sensitivity and specificity may seem high enough for the test to be of use. That is because the number of false positives, in this case 49, is significantly higher than the total number of people with the underlying condition, in this case 20. With a PPV of 26.9% the test would be of no practical use, because the diagnosing physician could only say with a 26.9% probability that the patient had the condition.

The following example shows what happens if the prevalence is increased to 10% without changing the other parameters:

	condition	not condition	tot
+ve	90	45	135
-ve	10	855	865
tot	100	900	1000

PPV = 90/(90+45) x 100 = 66.7%
NPV = 855/(10+855) x 100 = 98.8%

The PPV has risen significantly due to the increase in prevalence from 0.2% to 10%, but at 66.7% is arguably still not high enough to be of practical use as it does not give confidence that the diagnosis has a high probability of being correct. A higher sensitivity and specificity would be required to give such confidence at a prevalence of 10%.

2. RT-PCR testing for SARS-CoV-2

Nucleic acid testing and in particular RT-PCR has been established as the preferred test for SARS-CoV-2 under public health policies and guidelines.[1] PPV and NPV analyses apply to the diagnosis of SARS-CoV-2. The World Health Organisation's policy is that:

"*...disease prevalence alters the predictive value of test results; as disease prevalence decreases, the risk of false positive increases. This means that the probability that a person who has a positive result (SARS-CoV-2 detected) is truly infected with SARS-CoV-2 decreases as prevalence decreases, irrespective of the claimed specificity.*" [2]

Numerous factors affect the accuracy of the RT-PCR test, in the pre-analysis, RT and PCR steps.[3] Values of sensitivity quoted in the literature for the RT-PCR test for SARS-CoV-2 typically vary from around 75% to 100%,[4,5] but it should be noted that figures at the higher end may be obtained using unusually high cut-off values for cycle threshold value (Ct). The Ct value refers to the number of cycles required to amplify the starting material to an amount which is detectable. Each cycle represents a nominal doubling of the number of target sequences identified, modified by the PCR efficiency and other factors.

Normally Ct values above 35 are not considered valid because of the likelihood that they are due to contamination or non-replicating viral fragments. This is demonstrated in a study of 3,790 samples which tested positive for SARS-CoV-2,[6] which correlated the Ct value with infectivity as measured by the ability to grow out the sample in tissue culture. The results as stated were:

"*It can be observed that at Ct = 25, up to 70% of patients remain positive in culture and that at Ct = 30 this value drops to 20%. At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive.*"

Three further references are given in the study in support of this finding, which is also supported by Dr Fauci, who has stated, "If you get a cycle threshold of 35 or more, the chances of it being replication competent are miniscule".[7]

The effect of Ct cut-off value on sensitivity can be observed by comparing the sensitivities of seven commercial assays which varied from 77% to 100% based on a cut-off Ct value of 42.5.[4] If the cut-off Ct value is lowered to 35, then the sensitivities drop to between 54% and 77%.

Values reported for specificity tend to be higher, typically in the range of 95% to 100%.[8,9,10] However, in some of these studies it is clear there are problems with reproducibility, suggesting the high specificities reported are not easily achieved in practice. For example, in one study, of 45 samples which had previously tested positive using RT-PCR, only 8 (18%) tested positive in the initial test. Of 52 samples which tested positive in the initial test, only 29 (56%) tested positive in the second test.[8]

It could be interpreted that non-confirmation in the second test represents a loss of sensitivity from the first test to the second, or alternatively that it represents a lack of specificity in the first test, i.e. the samples which did not confirm the second time had originally tested positive due to something else. Depending on which assumption you make, the sensitivity or specificity reduces to 56% (29/52). The authors tested for loss of sensitivity due to storage but did not obtain statistically significant results, suggesting the second interpretation, a lack of specificity, is more likely.

These findings are consistent with other studies demonstrating the low reliability of RT-PCR in general. In one study of 8,240 patients, 42 types of virus were initially identified, but on further investigation it was found that 23 were due to contamination in laboratory reagents or from the surrounding environment.[11] In another study, a survey of PCR users showed that 65% of respondents had experienced failure to repeat their own results.[3] While highly experienced PCR users working under ideal laboratory conditions may consistently be able to obtain the high sensitivities and specificities reported, it is unlikely these high values would be widely obtained in real-world practice.

3. PPV and NPV Impacts in Various Situations

For the following case studies, PPV and NPV are calculated based on three cases of sensitivity and specificity: high, typical and low, as follows:

High: sensitivity = 99 %, specificity = 99%.
Taken as the maximum likely values in practice.

Typical: sensitivity = 70%, specificity = 95%
Following the example from the British Medical Journal used for illustrative purposes.[10]

Low: sensitivity = 54%, specificity = 56%
Using the rationale described in section 2 of this report.

(a) Worldwide

According to Worldometer on 8 March 2021 the total number of cases reported was 117,515,398 and the total number of tests carried out was 1,679,318,809.[12] In order to estimate the average incidence and prevalence over 2020 and 2021, the following assumptions are made:

1. The tests were carried out over a period of 14 months.
2. The average duration of a case was 0.5 months.
3. The average number of tests per person is 1.5 (i.e. every second person is re-tested once).

The average incidence works out to be 8,393,957 cases per month, and the tested population is 1,119,545,873. The prevalence can be calculated as incidence multiplied by duration as a percentage of the tested population, which gives 0.37%.

High:	PPV = 27.1%,	NPV = 100.0%
Typical:	PPV = 5.0%,	NPV = 99.9%
Low:	PPV = 0.5%,	NPV = 99.7%

These results show that with the low world prevalence of 0.37%, even under the most optimistic conditions of sensitivity and specificity, the PPV is too low to render the test results of any practical value. The NPV values are high. These findings suggest that, contrary to what is sometimes reported, positive test results are unlikely to be a true reflection of the underlying condition whereas negative test results are.

(b) Australia

According to Worldometer on 8 March 2020 the total number of cases reported in Australia was 29,046 and the total number of tests carried out was 14,663,141.[12] Using the same assumptions as above, the average incidence works out to be 2075 cases per month, and the tested population is 9,775,427. The prevalence works out to be 0.011%.

Using the same values of sensitivity and specificity for the three cases as above, the PPVs and NPVs for Australia are:

High:	PPV = 1.0%,	NPV = 100.0%
Typical:	PPV = 0.15%,	NPV = 100.0%
Low:	PPV = 0.013%,	NPV = 100.0%

Clearly, at such a low prevalence, the PPV of the RT-PCR test for SARS-CoV-2 in Australia is so low as to render the test results of no value. Almost all positive test results would be false positives, whereas negative test results are, to one decimal place, 100.0% likely to be correct.

(c) Victorian "snap" lockdown

During February 2021, Victoria was placed on a snap lockdown based on five new cases of SARS-CoV-2, taking the total number of cases to 20,465.[13] The total number of tests carried out at the time was 4,652,545. Assuming testing had been carried out over 13 months, an average duration per case of 0.5 months, and an average number of tests per case of 1.5, the average incidence is 1,574 cases per month, and the average prevalence is 0.025%.

The PPVs and NPVs corresponding to the three levels of sensitivity and specificity are shown below:

High:	PPV = 2.5%,	NPV = 100.0%
Typical:	PPV = 0.35%,	NPV = 100.0%
Low:	PPV = 0.03%,	NPV = 100.0%

At the low prevalence of 0.025% these results for PPV are too low to render the test results of any value. Even with a high assumed sensitivity and specificity, they had a 97.5% chance of being wrong.

(d) Pfizer safety and efficacy study

According to the Pfizer mRNA Covid-19 Vaccine trial published in the New England Journal of Medicine on 31 December 2020, the efficacy calculations were based on a total of 170 cases.[14] Eight cases came from the vaccinated group and 162 from the control group. The total number of at-risk participants were 17,411 and 17,511 respectively. The average surveillance time is calculated from Table 2 to be 1.5 months. Assuming an average duration per case of 0.5 months, the incidence for the vaccinated and control groups works out to be 5.3 and 108 cases per month respectively. These numbers convert to prevalences of 0.015% and 0.31% for the vaccinated and control groups respectively.

The PPV and NPV results for the vaccinated group are:

High:	PPV = 1.5%,	NPV = 100.0%
Typical:	PPV = 0.21%,	NPV = 100.0%
Low:	PPV = 0.019%,	NPV = 100.0%

The low PPV values indicate the test results are of no practical value for the vaccinated group, even at high levels of sensitivity and specificity.

The results for the control group are:

High:	PPV = 23.4%,	NPV = 100.0%
Typical:	PPV = 4.15%,	NPV = 99.9%
Low:	PPV = 0.38%,	NPV = 99.75%

The PPV values in the control group are higher than in the vaccinated group due to the higher assumed prevalence, but even at the highest levels of sensitivity and specificity the PPVs are too low for the test results to be of practical value.

(e) AstraZeneca safety and efficacy study

The AstraZeneca safety and efficacy study was published in the Lancet on 8 December 2020.[15] For all low dose and high dose participants in the vaccinated groups 30 positive cases were reported out of a total of 5,807 participants. In the control group 101 positive cases were reported out of 5,829 participants. The average surveillance time is calculated from Table 2 to be 1.4 months. Assuming an average duration per case of 0.5 months, the incidence for the vaccinated and control groups works out to be 21.4 and 72.1 cases per month respectively. These numbers convert to prevalences of 0.18% and 0.62% for the vaccinated and control groups respectively.

The PPV and NPV results for the vaccinated groups are:

High:	PPV = 15.5%,	NPV = 100.0%
Typical:	PPV = 0.68%,	NPV = 100.0%
Low:	PPV = 0.06%,	NPV = 100.0%

In all cases the PPVs indicate the test results are of no practical value for the vaccinated group.

The results for the control groups are:

High:	PPV = 38.1%,	NPV = 100.0%
Typical:	PPV = 8.0%,	NPV = 99.8%
Low:	PPV = 0.76%,	NPV = 99.5%

In all cases the PPVs indicate the test results are of no practical value for the control group.

4. Conclusion

Published values for sensitivity and specificity for RT-PCR tests for SARS-CoV-2 have been obtained, and positive predictive values based on three assumed levels of sensitivity and specificity have been estimated for certain groups including the world, Australia, Victoria, and the Pfizer and AstraZeneca trial cohorts. Although the negative predictive value remains high, in all cases the prevalence is too low to result in PPV values high enough for the test results to be of any practical value.

PPV is an important parameter in testing for medical conditions as it gives an indication of the reliability of test results, yet it appears to have been overlooked when evaluating the data arising from the implementation of mass testing. When PPV values are taken into account, they show that the use of mass testing in low-prevalence settings is of no practical value and cannot be justified, because the probability that the results are correct is too low for them to be of any value.

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