

## Potential false-positive rate among the 'asymptomatic infected individuals' in close contact with COVID-19 patients

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### Abstract:

**Objective** As the prevention and control of COVID-19 continues to advance, the active nucleic acid test screening in the close contacts of the patients has been carrying out in many parts of China. However, the false-positive rate of positive results in the screening has not been reported up to now. But to clarify the false-positive rate during screening is important in COVID-19 control and prevention.

**Methods** Point values and reasonable ranges of the indicators which impact the false-positive rate of positive results were estimated based on the information available to us at present. The false-positive rate of positive results in the active screening was deduced, and univariate and multivariate-probabilistic sensitivity analyses were performed to understand the robustness of the findings.

**Results** When the infection rate of the close contacts and the sensitivity and specificity of reported results were taken as the point estimates, the positive predictive value of the active screening was only 19.67%, in contrast, the false-positive rate of positive results was 80.33%. The multivariate-probabilistic sensitivity analysis results supported the base-case findings, with a 75% probability for the false-positive rate of positive results over 47%.

**Conclusions** In the close contacts of COVID-19 patients, nearly half or even more of the 'asymptomatic infected individuals' reported in the active nucleic acid test screening might be false positives.

**Key words:** COVID-19 Close contacts Nucleic acid test Screening False-positive

With the continuous spread of the COVID-19 epidemic prevention and control work, localities in China have strengthened the monitoring of confirmed and suspected cases in accordance with the recommendations of the National Health and Health Commission's "New Coronavirus Pneumonia Prevention and Control Plan (Fourth

Edition)” (the “Plan”). Nucleic acid testing (real-time fluorescence RT-PCR) is performed for those who have any symptoms such as fever, chills, dry cough, etc. during contact isolation and observation. Many regions have also expanded this into proactive screening of close contacts without symptoms, and some regions have even expanded it to contacts of contacts (also known as "second-generation contacts"). Among the patients without any contact history and workers returning to work, the source of infection (asymptomatic infection) is found as soon as possible and quarantine is mandated, and close contacts are quarantined for medical observation.

## Control and extinguish local epidemics.

This active screening for nucleic acid testing will indeed play a positive role in controlling the local epidemic. However, even if it is a highly sensitive and highly specific detection method, which has good diagnostic value in clinical diagnosis of suspected cases, the screening results for the general population may not be ideal. The reason is that the general population has a low prevalence (or infection rate), and the prevalence directly affects the size of the positive predictive value, and the two have a positive correlation. This study aims to analyze possible false positives from active screening of secondary contacts of patients with COVID-19 cases, based on known information, estimates the reasonable range of relevant indicators, and deduces the proportion of false positives in the positive results of active screening measures. (i.e., positive predictive value), to provide a scientific basis for epidemic prevention and control, and to ensure that arrangements for prevention and control are reasonable.

## Materials and methods

1. Relevant information acquisition: We obtained relevant information by consulting official publications, the published scientific literature, expert reviews published online, disease control personnel and clinicians, and reviewed test data from individual laboratories.

(1) Basic conditions of the current real-time fluorescent RT-PCR detection method and the positive report of the positive detection results: According to the “Protocol”, confirmed cases must have laboratory-specific testing evidence, that is, “respiratory or blood samples positive in real-time fluorescent RT-PCR for the new coronavirus nucleic acid” or “respiratory or blood specimen virus gene sequencing, highly homologous to the known new coronavirus”. Because the second method requires stringent experimental conditions and technology, and has a long waiting time, the real-time fluorescent RT-PCR detection method is currently widely used in most laboratories<sup>[1]</sup>. The “Proposal” states that this method is mainly aimed at the open reading frame 1ab (ORF1ab) and the nucleocapsid protein N gene in the 2019-nCoV genome. In the laboratory, it is necessary to determine that the sample is positive for both targets’ (ORF1ab and N) specific RT-PCR. The “Protocol” (Fifth Edition), released on February 22,

2020, no longer requires that a test must meet these conditions to be considered positive. Instead, alternative conditions have been added, suggesting that the stringency of the positive test criteria is decreasing to improve the sensitivity of the test. On the basis of guaranteeing the two targets required by the Protocol, some kit manufacturers have also added other targets such as envelope protein E and S genes to assist laboratory diagnosis. However, in the kit instructions, the positive determination standard still complies with the requirements of the “Protocol”.

Theoretically, if the timing and quality of the specimen collection are appropriate, the specimens are transported, received, and processed in a standardized manner, laboratory equipment and technology meet the standards, and the detection process (nucleic acid extraction, nucleic acid amplification, positive and negative control comparison, etc.) is strictly in accordance with the kit instructions, the method of real-time fluorescent RT-PCR detection should have high sensitivity and specificity <sup>[1-2]</sup>. However, in actual operation, non-ideal effects may occur at each step, leading to doubts about the accuracy of the test results, and producing false negative and false positive results. In particular, poor quality of the collected samples will greatly reduce the sensitivity of the test <sup>[1]</sup>. The COVID-19 epidemic has created time urgency, which means that the kits developed by various manufacturers were unable to complete the normal clinical verification and evaluation process, especially the verification of a certain number of clinical patient specimens <sup>[1]</sup>. The accuracy and reliability of the results obtained with these kits have not been evaluated at present, and only one report has been published for different types of specimens with confirmed cases <sup>[3]</sup>.

Although the “Proposal” does not explicitly require a positive initial test to be re-tested to confirm the positive report, due to careful consideration of confirmed case reports, at present, the results of the initial test are typically reported immediately if positive, and negative results of the initial test are not. In this case the person will be re-tested, and in this case, both the positive or negative second test result will be reported.

(2) Information supporting the estimation of relevant indicators: The factors that affect the size of the positive predictive value include the prevalence (infection rate) of the test population and the sensitivity and specificity of the test method.

For close contacts who have not yet developed symptoms, the prevalence of 2019-nCoV infection should be relatively low, especially in non-epidemic areas. This study learned from the epidemic prevention and control headquarters in a non-epidemic area that out of 5,165 close contacts (more than 2,000 of which have not been released from quarantine) of the cumulative confirmed cases and suspected cases registered as of February 22, only 49 people were

diagnosed as confirmed (all had symptoms and positive test results), accounting for 0.949%; and another 17 were confirmed as “asymptomatic infection” (positive test result but no symptoms), accounting for 0.329%. The test result was therefore positive for less than 2 out of every 100 people tested. The situation in the other two non-epidemic areas surveyed was largely the same. It is estimated that the proportion of “asymptomatic infections” reported by active screening among close contacts of confirmed cases and suspected cases is about 1%. Seven China CDC personnel from four non-epidemic areas were consulted, and they all agreed that the highest rate of asymptomatic positives was not more than 10%, and the lowest was definitely under 1%.

Considering the sensitivity and specificity of the detection method, laboratory personnel report that the detection method itself is trustworthy. Regardless of the quality of the kit and the aforementioned factors that affect the accuracy of the test results, the sensitivity and specificity of the single-time real-time fluorescent RT-PCR test method should be in accordance with the 2019-nCoV positive determination criteria specified in the “Protocol”, i.e. > 90% accurate. However, what is discussed here is the final confirmation of the reported sensitivity and specificity based on actual work and detection results. This study does not consider possible quality problems in the process from sample collection to the final test result, nor does it consider the possible quality problems of kits produced by various manufacturers, and only considers the type of samples that were used. Contacts of coronavirus cases who are actively screened may have no symptoms and therefore only upper respiratory tract specimens (pharyngeal swabs, nasal swabs, nasopharyngeal extracts, etc.) can be collected for nucleic acid testing. Even if the sampling meets the specifications, the amount of virus in some infected people may be too small to be detected <sup>[1-2]</sup>, which will reduce the sensitivity of the test. Just as the suspected cases reported in many places were tested after several samplings (including the collection of lower respiratory tract specimens) before the final diagnosis was confirmed.

After consulting 6 clinicians and laboratory personnel in 4 non-epidemic areas, the feedback information was summarized, and the sensitivity of a single real-time fluorescent RT-PCR test in actual work was estimated to be 50% to 70% (including the diagnosis of suspected cases). As a result of the preliminary inspection and re-examination of double-positive procedures for reporting positive results for some specimens in actual work, this measure will increase the specificity of the test and reduce the sensitivity of the test. Based on the judgment of the above laboratory personnel and the actual work situation, in addition, this study found from individual CDCs that the congruence of positive retests with the initial test is 80% ~ 90% (the rate increases with an increase in job proficiency). This indicates that false-positive test results do exist, and the specificity reported in this study is estimated to be around 95%.

2. Estimation of the infection rate and reported sensitivity and specificity of close contacts of active screening: Based on the information obtained above, this study estimates the 2019-nCoV infection rate of actively screened close contacts, the reported sensitivity and specificity, and is shown in Table 1.

**Table 1. Point and interval estimates of infection rates and reported sensitivity and specificity of close contacts who were actively screened**

Parameter	Point Estimate	Interval Estimate
Rate of infection	2%	1-10%
Sensitivity	60%	50-70%
Specificity	95%	90-99%

In order to conservatively estimate the proportion of false positives in the positive results of active screening, this study raised the estimated infection rate of close contacts to 2%, and adjusted the upper limit of its reasonable interval to the maximum probability of 10%. In addition, keeping the sensitivity in the range of 50% to 70%, we did not consider the case where the process of reporting a positive result before the preliminary inspection and double inspection will reduce sensitivity; we adjusted the upper limit of the reasonable specificity interval to a maximum value of 99%.

Data analysis: First we calculated the positive predictive value and the false positive ratio of the positive results of the screening based on the infection rate, sensitivity, and specificity indicators under the point estimates to obtain the most likely results (baseline results). Subsequently, a single-factor sensitivity analysis was performed, that is, each index affecting the positive predictive value was individually changed within its own reasonable interval to see the change in the proportion of false positives in the positive results. Finally, a multi-factor probabilistic sensitivity analysis (Monte Carlo simulation) was performed to allow the three indicators that affect the positive predictive value to be randomly selected according to their respective distributions within their respective reasonable intervals. 100,000 times, the median, quartile, minimum, and maximum values of the 100,000 simulation results were calculated and expressed in a box diagram. Because it is currently impossible to determine the distribution of each of the three indicators that affect the positive predictive value, for the sake of caution, this study uses a simple triangular distribution and a uniform distribution to assume the distribution of the three indicators. In the sensitivity analysis, this study does not consider the negative correlation between the sensitivity and specificity indicators.

## Results

1. Baseline results: When the infection rate, sensitivity, and specificity are estimated by point values, assuming that 1,000 people are screened, the calculated positive predictive value and the false positive ratio of the positive results are shown in Table 2.

**Table 2 positive predictive value of screening and false positive proportion in positive results when point estimations are taken for all three indicators**

Test results	Actually positive	Actually negative	Total	PPV	False positives
Positive	12	49	61	19.67%	80.33%
Negative	8	931	939		
<b>Total</b>	<b>20</b>	<b>980</b>	<b>1,000</b>		

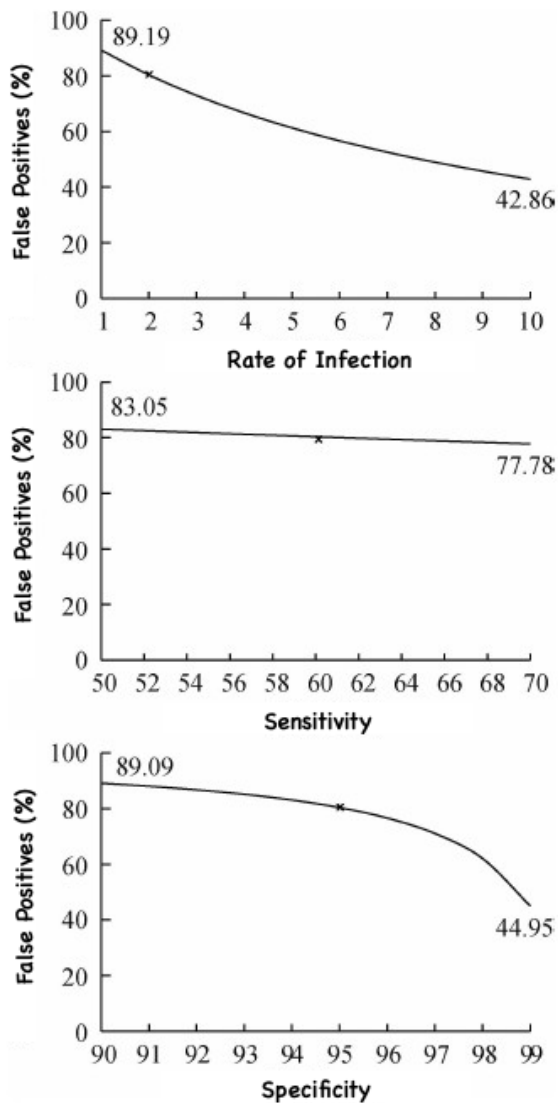
### Notes

Positive Predictive Value (PPV) =  $12/61 = 19.67\%$

False Positives =  $49/61 = 80.33\%$

2. Sensitivity analysis results: The results of single-factor sensitivity analysis showed that a single change in sensitivity within a reasonable interval had little effect on the proportion of false positives in positive results (from 83.05% to 77.78%). However, independent changes in the two indicators of infection rate and specificity within their respective reasonable intervals have a great impact on the proportion of false positives in positive results: as the infection rate increases from 1% to 10%, the proportion of false positives in positive results gradually decreases from 89.19% to 42.86%; as the specificity increased from 90% to 99%, the proportion of false positives in the positive results decreased slowly from 89.09%, and then rapidly decreased to 44.95%. No matter which indicator changes independently within a reasonable interval and takes any value, the proportion of false positives in positive results will always be greater than 40%. see Figure 1.

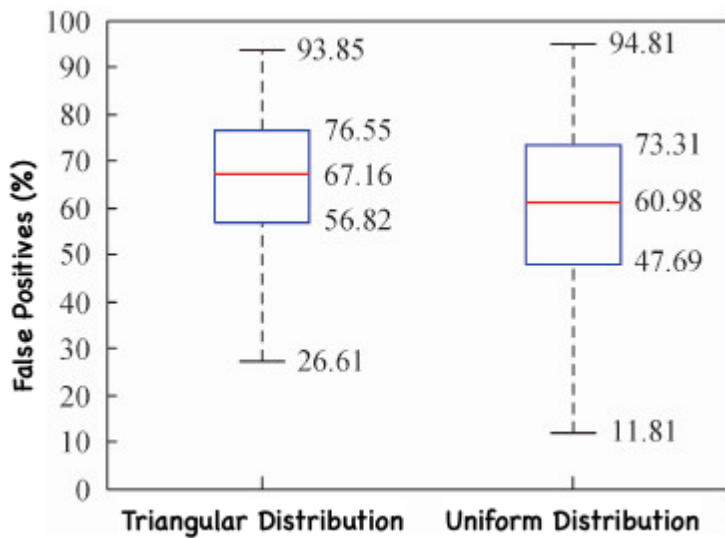
**Figure 1: Sensitivity Analysis Results**



Note: \* represents the reference result,

The results of the sensitivity analysis of the three indicators (Figure 2) show that when all three parameters are triangularly distributed, the median proportion of false positives in 100,000 simulated positive results is 67.16%, and the quartiles are 56.82% and 76.55%, the minimum and maximum are 26.61% and 93.85% respectively. When the three indicators are more conservative and uniformly distributed, the median (60.98%) and quartile (47.69% and 73.31%) decrease, but the range increases. It is suggested that the probability of false positives in the positive results is greater than 47% and the probability is 75%.

**Figure 2: Multivariate Probability Sensitivity Analysis Results**



## Discussion

With the increasing number of manufacturers of COVID-19 nucleic acid detection kits, the production has met the needs testing laboratories, the responsibility for case diagnosis has been widened, and prevention and control work is covering more areas. Nucleic acid testing has become an active screening measure in many regions. The types of people targeted for screening has also been widened, which will undoubtedly result in the reporting of more and more “asymptomatic infections”. Based on the information currently available, this study estimates the reasonable range of changes in related indicators and deduces the proportion of false positives in positive screening measures among close contacts of the case.

This study found that when the infection rate and reported sensitivity and specificity of close contacts were taken as the most probable values, the positive predictive value was only 19.67%. Conversely, the proportion of false positives in the positive results was 80.33%. The results of multivariate probability sensitivity analysis showed that the probability of false positives being greater than 47% in positive results was 75%, suggesting that among close contacts of COVID-19 cases, in cases of “asymptomatic infection” found by active nucleic acid testing there may be half or even more false positives. The univariate sensitivity analysis found that the reported sensitivity alone had a small effect on the results within a reasonable interval, but if the specificity of the test became greater than 98%, it significantly reduced the proportion of false positives in the positive results. Also, in places where the infection rate is 10% and not 1%, it will also significantly reduce the proportion of false positives, suggesting that to clarify the positive predictive value of active screening or the proportion of false positives in positive results, it is necessary to conduct in-depth research on specificity and infection rate indicators to obtain accurate data.



The results of this study are intended to remind staff involved in epidemic prevention and control that they should be alert to the fact that almost all detection techniques and methods are not 100% accurate. According to the results of the study, in view of the current situation of epidemic prevention and control, this study does not recommend the widespread screening using nucleic acid tests among second-generation contacts, general clinicians and workers returning to their jobs, especially in non-epidemic areas. These populations are less likely to be infected, and extensive screening will likely be false-positive even if a few “asymptomatic infections” are also found. In addition to causing an unnecessary waste of resources and impact on the lives and work of those who test false positive, and their close contacts, it will also disperse energy and affect the focus of prevention and control, this study also does not recommend the active screening using nucleic acid detection even for those who are in close contact with a confirmed case. It should focus on close contacts who have been exposed for a long time or have had multiple exposures in families, hospitals, confined spaces and other environments, to improve active the efficiency of screening.

With the in-depth study of 2019-nCoV, people have realized that there may be a large proportion of asymptomatic infections, and that those with asymptomatic infection may be infectious, which is a big challenge for epidemic prevention and control. The results of this study suggest that a higher percentage of false positives may be reported among asymptomatic infections reported in various regions (e.g. the 17 “asymptomatic infections” mentioned in the data and methods, in which at least one repeated nucleic acid test was performed, 3 people tested positive again, but the other 14 were negative, and not one of them has yet become a confirmed case). To clarify the status of these cases, nucleic acid testing needs to be supplemented by the results of future serological tests, especially a population epidemiological survey.

### Conflict of interest:

All authors declare that there is no conflict of interest

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