

# Combined Diagnosis of SARS-CoV-2: Rapid Antigen Detection as an Adjunct to Nucleic Acid Detection

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**Abbreviations:** RT-PCR, reverse transcriptase–polymerase chain reaction; RAD, rapid antigen detection; WHO, World Health Organization; NAAT, nucleic acid amplification test; ORFs, open reading frames, S, spike, E, envelope, N, nucleocapsid; Ct, cycle threshold; POCT, point-of-care testing; LFIA, lateral flow immunoassay, ECLIA, electrochemiluminescence immunoassay, CLIA, chemiluminescence immunoassay, CLEIA, chemiluminescence enzyme immunoassay, ELISA, enzyme-linked immunosorbent assay.

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## ABSTRACT

Coronavirus disease 2019 is a serious threat to human life, and early diagnosis and screening can help control the COVID-19 pandemic. The high sensitivity of reverse transcriptase–polymerase chain reaction (RT-PCR) assay is the gold standard for the diagnosis of COVID-19, but there are still some false-negative results. Rapid antigen detection (RAD) is recommended by the World Health Organization (WHO) as a screening method for COVID-19. This review analyzed the characteristics of RDT and found that although the overall sensitivity of RAD was not as high as that of RT-PCR, but RAD was more sensitive in COVID-19 patients within 5 days of the onset of symptoms and in COVID-19 patients with Ct ≤ 25. Therefore, RAD can be used as an adjunct to RT-PCR for screening patients with early COVID-19. Finally, this review provides a combined diagnostic protocol for RAD and nucleic acid testing with the aim of providing a feasible approach for COVID-19 screening.

As of March 6, 2022, more than 433 million confirmed cases and more than 5.9 million deaths have been reported worldwide for COVID-19 caused by SARS-CoV-2.<sup>1</sup> The spread of COVID-19 can be effectively controlled by extensive screening, close contact tracing, and isolation

of infected individuals. Currently, the nucleic acid amplification test (NAAT) is still the gold standard for COVID-19 diagnosis, and RT-PCR is mostly used<sup>2</sup>; however, NAAT has some disadvantages, such as strict testing environment, high personnel and technical requirements, expensive instruments and reagents, and long testing time, etc. Moreover, NAAT has false negatives and false positives, which may lead to a certain probability of wrong and missed tests. The World Health Organization (WHO) and some countries have issued guidelines for the use of rapid antigen detection (RAD).<sup>3–5</sup> The RAD is relatively simple and inexpensive to perform, can be performed without trained specialists or specialized instruments, and can report and interpret results in less than 30 minutes. However, RAD also has shortcomings, such as lower sensitivity. Therefore, either method alone cannot simultaneously maximize the accuracy or efficiency or minimize the cost of SARS-CoV-2 detection. The RAD, if used as an adjunct to COVID-19 diagnosis, will contribute to the accurate diagnosis of COVID-19 in combination with NAAT methods and play a crucial role in the control of the COVID-19 pandemic. This review analyzes the characteristics of RAD and RT-PCR testing and provides a combined testing protocol intending to provide a feasible method for COVID-19 screening in different populations.

## Nucleic Acid Testing

About two-thirds of the 5'-terminal of the SARS-CoV-2 genome is composed of overlapping open reading frames (ORFs) ORF1a and ORF1b, which are mainly responsible for encoding nonstructural proteins such as enzymes related to viral replication and transcription. The other one-third of the genome encodes major structural proteins such as spike (S) protein, envelope (E) membrane protein, membrane protein, and nucleocapsid (N) protein. The structural proteins determine the replication, stability, and invasiveness of the virus.<sup>6</sup> The S protein on the surface of SARS-CoV-2 specifically recognizes the angiotensin-converting enzyme 2 receptor on the host cell membrane and mediates the binding of the virus to the host cell membrane. The SARS-CoV-2 stimulates the body's defense system during viral replication, amplification, and release. Persons with COVID-19 fight the virus through autoimmune function and adjuvant therapy against inflammation.<sup>7</sup> However, an over-activated inflammatory response and cytokine storm may trigger viral pneumonia. There is no specific treatment for COVID-19; therefore, early diagnosis and timely prevention are the keys to controlling the outbreak. The gold standard for SARS-CoV-2 diagnosis is RT-PCR, the principle of which is to monitor the growth of the number of products in real time by specific

fluorescent-labeled probes and calculate the initial template amount based on the amplification curve. The kits are designed with specific primers for the detection of SARS-CoV-2 against the ORF1ab fragment and the N gene, and the diagnosis of positive cases requires 2 positive targets in the same specimen. A study optimizing the detection of SARS-CoV-2 suggested the use of PCR targeting the E gene followed by confirmation using RNA-dependent RNA polymerase primers in combination with SARS-CoV-2-specific probes.<sup>8</sup>

The number of copy amplification cycles recorded by RT-PCR is the cycle threshold (Ct) value, and the critical level for Ct value for RT-PCR is generally set at 37 to 40 globally and results greater than the critical value are considered negative.<sup>9</sup> The Ct value threshold for RT-PCR varies from laboratory to laboratory due to differences in population, geography, instrumentation, and reagents. The Ct value is widely used as a semi-quantitative indicator of SARS-CoV-2 viral load; a lower Ct value corresponds to higher severity of the disease.<sup>10</sup> In contrast, asymptomatic infected patients may have higher Ct values leading to false-negative results.<sup>11</sup> Therefore, the sensitivity of RT-PCR in asymptomatic infected patients needs to be improved. The RNA count of COVID-19 pharyngeal virus peaks on the fourth day of symptom onset,<sup>12</sup> One study found that the false-negative rate of RT-PCR decreased gradually to a minimum from the day of infection to the third day of symptom onset and then increased gradually on the fourth day of symptom onset.<sup>13</sup> In addition, RT-PCR assay results are also susceptible to sampling site effects, with the following viral loads for different specimen types: alveolar lavage > deep cough sputum > nasopharyngeal swab > oropharyngeal swab > blood.<sup>14</sup> Currently, nasopharyngeal swabs and oropharyngeal swab sampling methods are most common. Therefore, early in the patient's infection, low nasopharyngeal and oropharyngeal viral loads lead to an increased likelihood of false-negative RT-PCR results.

The RT-PCR method also has some obvious disadvantages: (i) the test requires sophisticated instruments, which are usually expensive and limited in number, and the population in need cannot perform point-of-care testing (POCT) on their own; (ii) testing personnel and the population in need must go to a designated location for professional specimen collection, which may lead to clustering of infections; (iii) the assay takes longer, the experiment itself requires a certain amount of cycle time, and in the case of relatively large numbers of samples, the test needs to be performed in batches. In addition, patients with the virus may still spread the virus while the results are not reported. During the SARS-CoV-2 pandemic, new variants of the virus have emerged, such as the Delta and Omicron variants. The Delta variant is more transmissible, has an increased viral load, is more pathogenic, and has a longer duration of the transmission.<sup>15</sup> The Omicron variant was first identified in southern Africa in November 2021. The Omicron variant has a greatly enhanced immune escape and infectious ability, resulting in the rapid global spread of the Omicron variant.<sup>16</sup> Due to the emergence of variant strains such as these, existing probes may be insensitive, resulting in false-negative results. Numerous reports have evaluated nucleotide sequence variants in RT-PCR primers and probes that produce mismatches and may lead to false-negative results.<sup>17</sup>

## RAD

The WHO recommends RAD that meets the minimum performance requirements of  $\geq 80\%$  sensitivity and  $\geq 97\%$  specificity. Antigen detection mainly targets the N-protein antigen locus of SARS-COV-2, and the overall sensitivity of RAD is lower than RT-PCR, so RAD cannot replace

RT-PCR as a diagnostic method for COVID-19. However, RAD also has certain advantages, such as short detection time and the possibility of POCT. Therefore, RAD can be used as an auxiliary method to improve the diagnostic efficiency of COVID-19 in combination with RT-PCR. Most of the RAD tests are performed using nasopharyngeal swabs, oropharyngeal swabs, or bilateral anterior nasal swabs, and the detection methods include both qualitative and quantitative assays. Qualitative assays are mainly performed by lateral flow immunoassay (LFIA): 2 horizontal lines appear on the membrane, 1 in the quality control (QC) area and the other in the test area. The color intensity of the horizontal lines in the test area may vary depending on the concentration of SARS-CoV-2 antigen in the sample. The presence of a horizontal line in both the test and QC areas is considered a positive result, and the presence of a horizontal line in the QC area only is considered a negative result. If no horizontal line appears in the QC area, it is an invalid result. Generally, the test result can be determined as negative or positive by visual inspection within 15 to 20 minutes, and the test method is easy to learn. Quantitative assays mainly use automated antigen detection, which requires specific detection instruments and platforms that can detect specific antigen content.

## LFIA

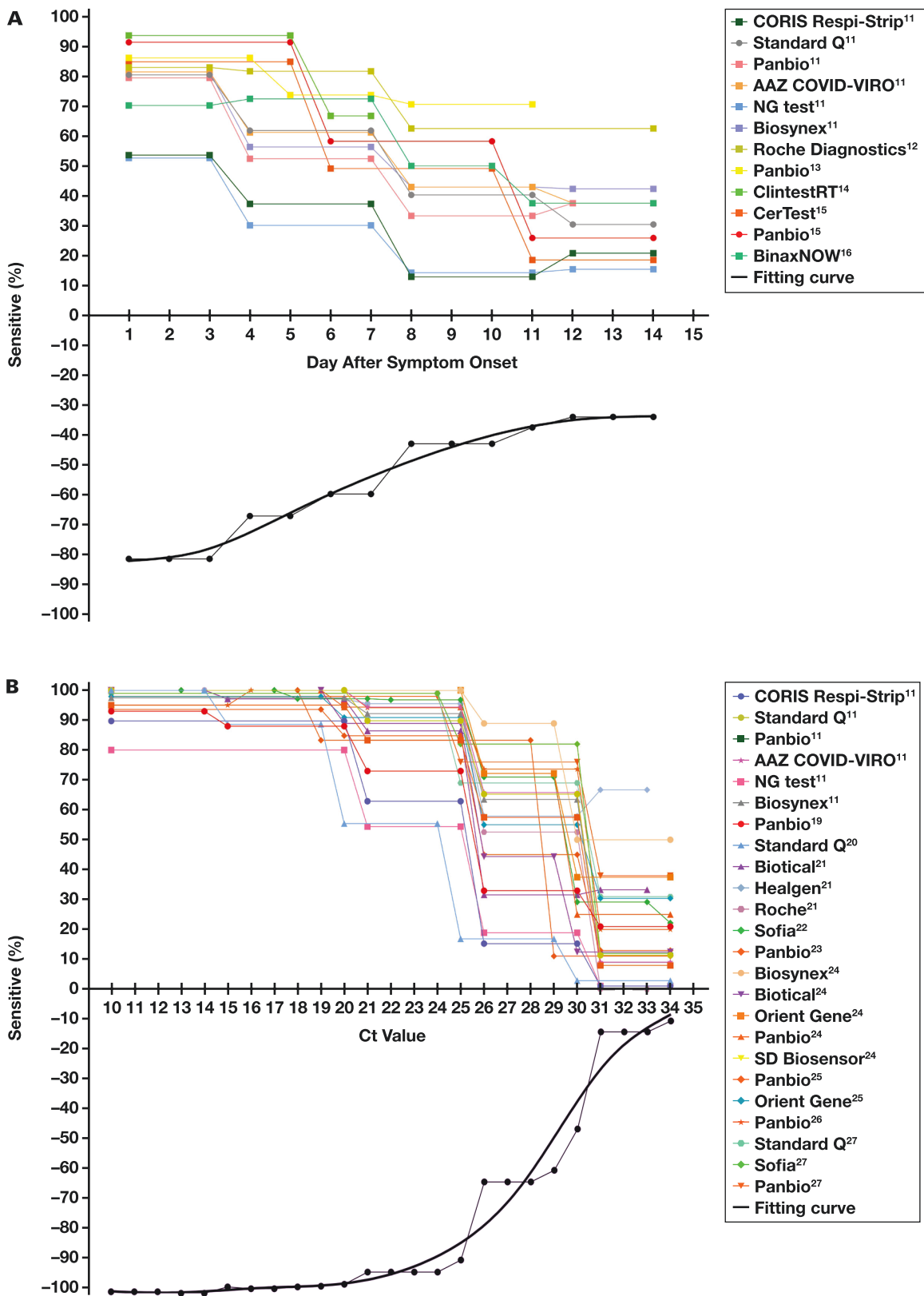
### ***LFIA Sensitivity and Number of Days to Symptom Onset in Persons with COVID-19***

Patients with COVID-19 often present with symptoms such as fever, cough, and weakness at the beginning of the infection. Many studies have performed LFIA tests on patients with COVID-19 at different days after the onset of symptoms and tracked the sensitivity of the tests on different periods. Various LFIA kits were used to test persons infected with SARS-CoV-2 and found that the sensitivity was generally lower than that of RT-PCR assays, with the highest sensitivity occurring at 1 to 3 days,<sup>18,19</sup> 1 to 4 days,<sup>20</sup> 1 to 5 days,<sup>21,22</sup> and 1 to 7 days.<sup>23</sup> As shown in **FIGURE 1A**, the first time-inflection point of the decrease in LFIA sensitivity occurred mostly within 5 days of symptom onset, the second time inflection point was on day 7 of symptom onset, and the third time inflection point was around 11 days after symptom onset. The results of the included studies were fitted to a curve showing that the sensitivity of LFIA was higher in the first 5 days and decreased significantly after 5 days. Therefore, the use of LFIA during this period has a high SARS-CoV-2 detection rate and can be used for self-testing and mass screening of COVID-19 when the person initially feels unwell; however, this method is not suitable for disease surveillance of persons with COVID-19 1 week after the onset of symptoms. In addition, a large proportion of persons with COVID-19 do not show any symptoms but are infectious. Frequent screening for SARS-CoV-2 in asymptomatic populations using antigen-based POCT has a very low detection rate and a high false-alarm rate.<sup>24</sup> Therefore, before using LFIA for COVID-19 screening, the characteristic distribution of the infected population, the accuracy of the LFIA kit, and the variation in sensitivity over time need to be carefully evaluated. When using LFIA for COVID-19 screening, it is necessary to increase the number of LFIA tests or combine LFIA with NAAT tests, especially in asymptomatic people and persons with symptomatic episodes >5 days.

### ***LFIA Sensitivity and Ct Values in Different Ranges of RT-PCR in COVID-19 Diagnosis***

Ct value is inversely proportional to viral RNA copy number<sup>25</sup>; a lower Ct value means a higher viral load. Subjects with COVID-19 were classified

**FIGURE 1 A**, Sensitivity of different LFIA kits in COVID-19 patients at different days after the onset of symptoms. **B**, Sensitivity of different LFIA kits for Ct values in different ranges of RT-PCR. The legend indicates the types of kits used in the different references.



and categorized according to Ct values derived from RT-PCR, and subjects with different Ct values were tested by LFIA. The study showed that the sensitivity of RAD detection varied at different viral loads. The phases with

higher sensitivity occurred in Ct < 15,<sup>26-28</sup> Ct < 18,<sup>29,30</sup> Ct < 20,<sup>18,31-33</sup> and Ct < 25.<sup>31,34</sup> As shown in **FIGURE 1B**, the sensitivity of LFIA is highest when the Ct value is ≤ 20. The first inflection point of sensitivity decrease

occurs at the Ct value of 19 to 20, the second inflection point occurs at the Ct value of 24 to 26, and the third inflection point occurs at the Ct value of 29 to 31. Fitting the results of the included studies to a curve showed that LFIA has good sensitivity in detecting COVID-19 in those with Ct  $\leq$  25, whereas persons with Ct values between 30 and 35 receive a high false negative. In a study testing 31 RAD kits, 26 of the 31 RAD kits had sensitivities above 80% for viral loads above  $10^6$  genomic copies/mL (Ct  $<$  25); 10 of the 31 RAD kits had sensitivities of 80% or higher for Ct values between 25 and 30, and the other 5 RAD kits had sensitivities only slightly below 80%; for viral loads  $\leq 10^6$  genomic copies/mL (Ct  $\geq$  25), all tests evaluated had sensitivities below 80%.<sup>35</sup>; this demonstrates the dependence of RAD on viral load. The LFIA is more suitable for detecting COVID-19 in persons with high viral load at the beginning of SARS-CoV-2 infection when there is a large amount of upper respiratory virus shedding. The sensitivity of the LFIA was reduced in asymptomatic infected individuals relative to the symptomatic population, but the sensitivity was significantly higher at Ct  $<$  20,<sup>36</sup> with sensitivity of 86% at Ct value  $<$  25.<sup>37</sup> In general, the use of LFIA in late presentation individuals and the asymptomatic infected population should take into account its lower sensitivity by using RT-PCR as a co-diagnostic method.

The LFIA has good recognition of high SARS-COV-2 load, and the severity of COVID-19 determines the viral load.<sup>38</sup> Therefore, LFIA helps to identify and predict the severity of the disease.

### Automated Antigen Assays

Currently, automated antigen assays are mainly used for the quantitative detection of the N antigen of SARS-CoV-2, and the detection principles can be broadly classified into four types: electrochemiluminescence immunoassay (ECLIA), chemiluminescence immunoassay (CLIA), chemiluminescence enzyme immunoassay (CLEIA), and enzyme-linked immunosorbent assay (ELISA).

The Roche Elecsys SARS-CoV-2 antigen assay uses ECLIA to quantify the N antigen in the sample. Roche Elecsys SARS-CoV-2 antigen detects nasopharyngeal swab specimens for SARS-CoV-2 infection status with an overall sensitivity of 65.85% and a specificity of 100%. When the cut-off value for antigen determination was set to  $>0.673$ , the sensitivity could be increased to 74.8%.<sup>39</sup> Using RT-PCR as a reference, the sensitivity was over 90% at Ct  $\leq$  30, and the sensitivity of the assay was higher within the first week of onset than after 1 week.<sup>40</sup>

The LIAISON SARS-CoV-2 antigen assay uses CLIA to quantify the N antigen in the sample. The overall sensitivity and specificity of the LIAISON SARS-CoV-2 antigen assay, using RT-PCR as a reference, were 75.33% and 100%, respectively. The overall sensitivity increased to 96.55% with high viral load (Ct  $<$  18.57),<sup>41</sup> and sensitivity was 91.1% for Ct  $\leq$  23, 89.8% for Ct  $\leq$  25, and 67.9% for Ct  $\leq$  33.<sup>42</sup> The LIAISON SARS-CoV-2 antigen assay has shown good performance in identifying SARS-CoV-2 infection in individuals with medium to high viral loads.

The VITROS SARS-CoV-2 antigen test uses CLEIA to quantify the N antigen in the sample. The sensitivity of VITROS SARS-CoV-2 antigen for detecting SARS-CoV-2 samples ranged from 72.0% to 100% within 1 week of symptom onset and decreased from 25% to 75% after 1 week, from 93.8% to 100% for Ct  $\leq$  30 and from 15.4% to 72.7% for Ct  $>$  30.<sup>43</sup> The Lumipulse G SARS-CoV-2 antigen assay provides another platform for quantification of N antigens in samples using ECLIA. The overall sensitivity and specificity of the Lumipulse G SARS-CoV-2 antigen assay were 84.0% and 89.1%, respectively, compared to the molecular assay, and showed 86.4% agreement with RT-PCR results.<sup>44</sup> In a study comparing the Roche and Lumipulse automated assays,<sup>45</sup> both antigen

tests were shown to accurately detect SARS-CoV-2 antigen in RT-PCR-positive samples with high viral loads. In addition, antigen levels were correlated with viral load and Ct values determined by RT-PCR. The performance of the Roche and Lumipulse antigen tests was nearly identical, indicating that both tests have high diagnostic accuracy up to 9 days after symptom onset, with a gradual decline after 9 days.

The N-antigen SARS-CoV-2 antigen test uses ELISA to quantify the N antigen in the sample. The overall sensitivity was 90.1% compared to RT-PCR and showed high agreement with RT-PCR results, with a negative correlation between antigen concentration and Ct values.<sup>46</sup>

Automated antigen detection is more sensitive than LFIA but the detection speed is not as fast as LFIA, and automated antigen detection requires specific instruments that cannot perform POCT; automated antigen testing is faster than NAAT and gives a specific viral load, but is still not as sensitive as NAAT and can be used as a prescreening tool but not as a replacement for NAAT.

### Sensitivity of RAD Detection in SARS-COV-2 Variants

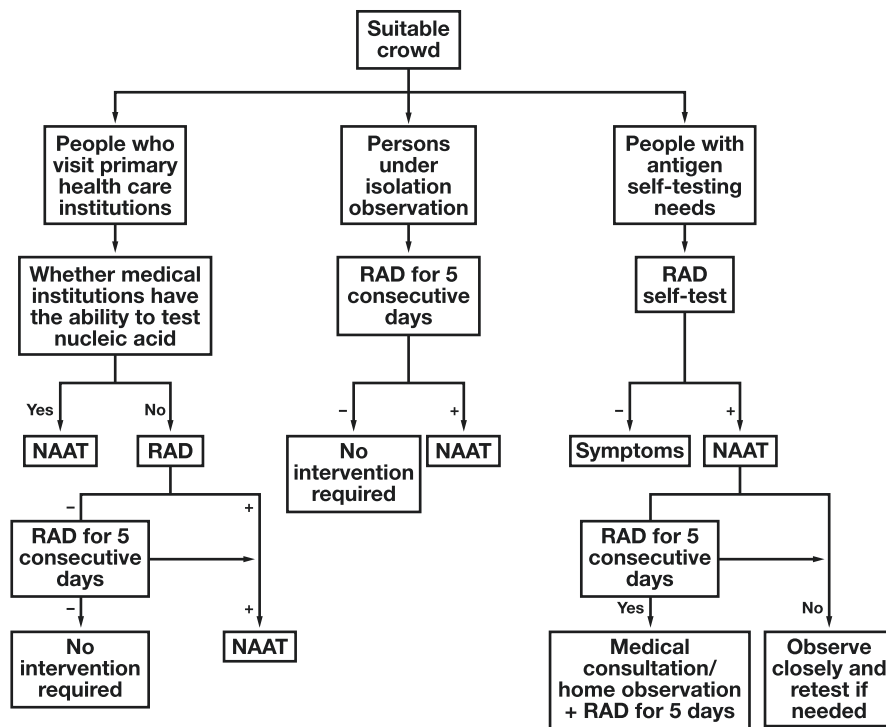
The COVID-19 mutation occurs mainly in the S protein of the virus, so other structural proteins, especially the N protein, can be better targets for detection due to the lower mutation rate. The newly detected Omicron variant contains only 2 mutations in the region encoding the N protein, whereas it contains more than 30 mutations in the S protein.<sup>47</sup> One study found reduced sensitivity of 9 antigen detection kits for both Delta and Omicron variants.<sup>48</sup> However, despite the slight difference in sensitivity, RAD is, in principle, effective in detecting Delta variants.<sup>49</sup> BinaxNOW detects high SARS-CoV-2 carriage during Omicron surge but should be repeated in high-risk populations with negative BinaxNOW results.<sup>50</sup> The RAD detected significantly fewer cases of COVID-19 with the Alpha variant than the Alpha nonvariant. This implies that the efficiency of antigen detection needs to be reevaluated in other areas where SARS-CoV-2 variants are predominant.<sup>51</sup> In the detection of nonvariant SARS-CoV-2, RAD showed a sensitivity of 90% ( $20 \leq$  Ct  $<$  25) and 10% ( $25 \leq$  Ct  $<$  30); In Beta or Gamma-associated SARS-CoV-2 variants, RAD has a detection sensitivity of 42.8% in samples with  $20 \leq$  Ct  $<$  25.<sup>52</sup> The marked decrease in sensitivity in SARS-CoV-2 variants suggests that special care must be taken when using RAD at the large-scale diagnostic level, especially in the current context of the emergence of several new SARS-CoV-2 variants that may produce false-negatives. The use of either RAD or RT-PCR alone increases false-negatives in the detection of SARS-CoV-2 variants, and the use of both RAD and nucleic acid diagnostics for SARS-CoV-2 can rapidly correct false-negative results and control and prevent COVID-19 outbreaks.

### Combined RAD and RT-PCR Protocol

The RAD detection site contains the bilateral anterior nostrils, and studies have shown that bilateral anterior nasal swabs have slightly lower or similar sensitivity than nasopharyngeal swabs.<sup>53,54</sup> The bilateral anterior nostril collection method caused significantly less severe coughing, sneezing, and pain and was more acceptable to the subjects than if the NAAT test site was the nasopharynx or oropharynx.<sup>55</sup> In addition, the combination of the 2 assays will increase the number of sites tested and reduce the number of missed diagnoses due to differences in viral load at the sampling site.

Different assays for RAD have different sensitivities, so the choice of a RAD as a prescreen for COVID-19 requires a thorough clinical

**FIGURE 2** Flow chart of the combined detection of SARS-CoV-2 using NAAT and RAD in different populations. NAAT, nucleic acid amplification test; RAD, rapid antigen detection.



evaluation in advance. The COVID-19 Outbreak Screening Centre in Hong Kong used a combination of RAD and RT-PCR methods to screen asymptomatic individuals through 2 processes.<sup>56</sup> In the first process, individual with initial positive RADs accelerated the testing of RT-PCR samples for these individuals by their laboratories, reducing the average time to confirm results from 10.85 hours to 7.0 hours. In the second process, individuals with an initial positive RAD undergo on-site rapid RT-PCR testing, reducing the average time to confirm results to less than 1 hour.

The population currently undergoing mass screening falls into 3 broad categories: (i) persons who visit primary health care institutions with respiratory and fever symptoms and have symptoms for 5 days or less; (ii) isolation observation personnel, including home isolation observation, close contact and subclose contact, inbound isolation observation, sealing control area, and control area personnel; (iii) community residents who have a need for antigen self-testing. Nucleic acid testing, when available, is preferred in the first category because of the presence of symptoms of COVID-19. The second and third categories are suitable for 5 consecutive days of self-testing with RAD. The testing process is shown in **FIGURE 2**. The RAD can play an important role in prescreening, and a positive RAD result must be confirmed with the gold standard (RT-PCR). Although using the 2 methods will improve the diagnostic efficiency and accuracy of COVID-19, the detection method should be continuously improved with probes and N-protein antibodies in response to the due to the continuous mutation of the strain.

## Conclusion

Due to some limitations of RT-PCR for SARS-CoV-2 detection, RAD can be used as an adjunct to RT-PCR. The overall sensitivity of RAD is

lower than that of RT-PCR, but it has high sensitivity and specificity in the early stage of SARS-CoV-2 infection. Therefore, RAD can be used as a screening tool for early SARS-CoV-2 infection, and RT-PCR used to confirm the diagnosis when the result is positive. There are many types of RADs on the market with varying test quality, and a thorough clinical evaluation should be performed before use. This review provides a flow chart for the combined application of RAD and NAAT to screen COVID-19 in the population, and it is believed that this protocol can effectively shorten the screening time for COVID-19 in a large base population and provide assistance in the control of the COVID-19 pandemic.

## Author Contributions

Xue-wen Li wrote the main parts of this manuscript and designed the figures; Yi-ting Wang wrote other parts and improved the language of the manuscript; Jian-cheng Xu conceived the structure and revised the manuscript; All authors read and approved the final manuscript.

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