

Analytical sensitivity of the Rapid Antigen Test kits for detection of SARS-CoV-2 Omicron variant BA.2 sublineage

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Abstract

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) Omicron was classified as a variant of concern in November 2021. The sublineage BA.2 spreads rapidly worldwide. Currently, there is a lack of data for the parallel comparison of Rapid Antigen Test (RAT) Kits to detect SARS-CoV-2 Omicron BA.2. We evaluated the analytical sensitivity of 12 RAT kits to detect Omicron BA.2 in the present study. Analytical sensitivity was determined by means of the limit of detection (LOD). We prepared a dilution set using a respiratory specimen collected from a COVID-19 patient infected by Omicron BA.2. The reverse transcription-polymerase chain reaction was used as a reference method. The LOD results showed that all 12 RAT kits had comparable analytical sensitivity to detect Omicron BA.2. The RAT kits selected in the current study may be used for the first-line screening of the rapid spreading Omicron BA.2.

KEYWORDS

B.1.1.529, BA.2, COVID-19, Omicron, Rapid Antigen Test, SARS-CoV-2

1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is continuously evolving. World Health Organization (WHO) classified SARS-CoV-2 as a variant of concern (VOCs) for those having global public health impacts. The latest VOC, Omicron was first detected in November 2021, it was classified as B.1.1.529 according to the PANGO nomenclature system.¹ The Omicron was divided into three sublineages, BA.1, BA.2, and BA.3.² Since then, different countries reported detection of Omicron cases. The latest global epidemiology of SARS-CoV-2 showed that Omicron became the dominating VOC with 99.9% prevalence.³

In Hong Kong, our lab detected the first case of Omicron in November, it belonged to BA.1.⁴ After that, we detected many Omicron cases and all of them were import-related. In late December 2021, local Omicron cases were detected. The Omicron BA.2 spread

rapidly in January 2022.⁵ The daily number of COVID-19 cases reported were remained over 10 000 after late February 2022.⁶

The gold standard to detect SARS-CoV-2 is reverse transcription-polymerase chain reaction (RT-PCR). Rapid Antigen Test (RAT) kits are alternative to RT-PCR due to the fast results and ease to use although these kits are inferior to RT-PCR in terms of sensitivity. RAT kits are useful to pick up high viral load cases. In Hong Kong, the testing strategy for COVID-19 cases has been revised in late February 2022. Suspected COVID-19 persons can self-perform RAT and register the positive results online. Those positive cases will be followed as usual without seeking confirmation by RT-PCR. With the support of Central Authorities, the government has procured a large number of RAT kits and provided them to different groups of people for free.⁷⁻⁹

The performance of RAT kits varied between different brands. However, there is a lack of data on the parallel comparison between RAT kits for the rapidly spreading Omicron BA.2.¹⁰ The purpose of

this evaluation is to assess the analytical sensitivity of RAT kits against Omicron BA.2.

2 | METHODS

2.1 | Respiratory specimen

The Public Health Laboratory Services Branch in Hong Kong has been designated as a WHO COVID-19 reference laboratory since April 2020 and aided in either diagnosing cases or confirming cases by referring hospitals/laboratories in Hong Kong.¹¹ To track variants, we have an intensive surveillance system to detect VOCs circulating worldwide.¹² The leftover respiratory specimens after RNA extraction were stored at -70°C . A respiratory specimen, combined nasopharyngeal and throat swabs, obtained from a COVID-19 patient collected on January 26, 2022 (hCoV-19/Hong Kong/VM22004564/2021) was selected for this evaluation. The genome sequence showed that it belonged to BA.2 lineage (GISAID accession number EPI_ISL_12335308). This specimen had a sufficient quantity ($>500\mu\text{l}$) and a high viral load (cycle threshold, $C_t < 20$) which fulfill the criteria for this evaluation.

2.2 | SARS-CoV-2 RAT kits

We routinely reviewed and evaluated RAT kits that were introduced to our lab by local suppliers.¹³⁻¹⁸ A total of 12 RAT kits were evaluated in the present study. The details of each kit were summarized in Supporting Information: Table S1. For ease of communication, these kits were coded arbitrarily from RAT-01 to RAT-12. RAT-01 to 11 were procured by Food and Health Bureau and Government Logistics Department which have not been evaluated by us between 2020 and 2021. Before this evaluation, we performed a screening for some RAT kits and found that they shared similar analytical performance when tested against the SARS-CoV-2 strain, hCoV-19/Hong Kong/VM22005395/2022. It belonged to BA.1 lineage and was obtained from a COVID-19 patient collected on January 29, 2022 (data not shown). The remaining kit, RAT-12 was procured by us, it has been evaluated in our previous rounds of evaluation.¹⁶⁻¹⁸

All of the kits evaluated were based on lateral flow principles. SARS-CoV-2 antibody was immobilized and coated on the test cassettes which can detect viral antigens. The test results can be interpreted by naked eyes. The test results were assessed and read by two technicians. Results grading of the band intensity were interpreted as previous.^{17,18} In case of doubtful results, the third technician interpreted the test results.

2.3 | Assessing analytical sensitivity of RAT kits

The dilution set of the specimen mentioned above was used to determine analytical sensitivity by means of the limit of detection

(LOD). The sensitivity of different kits can be obtained by measuring the lowest concentration of the specimen. To prepare the dilution set, serial tenfold dilution was performed from the stock of the specimen using phosphate-buffered saline as a diluent. As the stock of the specimen had a viral load, $C_t = 12.86$, only a few $100\mu\text{l}$ of the specimen was enough to prepare the dilution setting. In the beginning, a 100-fold dilution was performed for the first dilution point 10^{-2} . For example, it can be done by mixing $50\mu\text{l}$ of the specimen with $4950\mu\text{l}$ of the diluent. Then, serial tenfold dilution can be done by mixing $500\mu\text{l}$ of the new dilution point with $4500\mu\text{l}$ of the diluent. Based on this dilution approach, each dilution point consisted of enough quantity (i.e., $4500\mu\text{l}$) to test for all 12 RAT kits. Each dilution point was tested on the same day without a freeze and thaw cycle.

We employed a modified sample processing method to perform the RAT kits since we want to unify the input volume. Regarding the input volume, we previously found that RAT results were affected by the input volume. We evaluated two input volumes, 100 and $350\mu\text{l}$. The result bands were more intense when using $350\mu\text{l}$ specimen volume¹⁵ and this sample volume was selected for accessing LOD in the present study. The operating procedures were performed according to the manufacturer's instructions except first by mixing $350\mu\text{l}$ specimen volume with the kit's extraction buffer/diluent. Only one replicate was performed for each dilution point due to limited samples and a limited quantity of kits.

Virus concentrations in each dilution were estimated from the C_t value as described.¹³ Duplicates were performed for each dilution point and the C_t values shown were the mean of both runs.

3 | RESULTS

The LOD results for RAT kits against Omicron BA.2 were summarized in Table 1. All kits could detect dilution points 10^{-3} , the corresponding C_t value was 23.13. Five kits could detect dilution points 10^{-4} , the corresponding C_t value was 27.19.

The LOD for RT-PCR was 10^{-6} which was at least 100-fold more sensitive than the RAT kits.

4 | DISCUSSION

In the present study, our results showed that different RAT kits could detect Omicron BA.2 with similar analytical sensitivity. We employed standardized methods as before for evaluating RAT kits and hence, variations of other parameters such as specimen input volume and viral load quantification can be minimized.¹³⁻¹⁸

The SARS-CoV-2 VOCs are characterized by the S protein mutations. Most RAT kits target SARS-CoV-2 N protein. Unlike RT-PCR assays, the performance of different RT-PCR assays can be checked by aligning the sequences of primers and probes against different SARS-CoV-2 strains. It was impossible to check the performance of RAT kits against different SARS-CoV-2 strains as

TABLE 1 Comparison of the limit of detection for 12 Rapid Antigen Test (RAT) kits to detect SARS-CoV-2 Omicron BA.2

Dilution	RT-PCR	RAT kits ^a											
		RAT-01	RAT-02	RAT-03	RAT-04	RAT-05	RAT-06	RAT-07	RAT-08	RAT-09	RAT-10	RAT-11	RAT-12
10 ⁻²	19.62	++	++	++	++	++	ND	++	++	++	++	++	++
10 ⁻³	23.13	+	+	++	+	+	++	++	+	+	++	+	++
10 ⁻⁴	27.19	-	-	+	-	-	+	+	-	-	+	-	+
10 ⁻⁵	32.72	-	-	-	-	-	ND	-	-	-	-	-	-
10 ⁻⁶	35.88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10 ⁻⁷	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Abbreviations: ND, not done; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2; -, negative; +, weak positive; ++, positive.

^aThe details of each kit can be referred to Supporting information: Table S1.

the information regarding antibodies used for RAT kits was not available. The N protein of the SARS-CoV-2 Omicron BA.2 evaluated in the present study showed five mutations/changes, P13L, 31-33 deletion of ERS, R203K, G204R, and S413R when compared with the reference strain WIV04 (EPI_ISL_402124). Although we do not know if the RAT kits evaluated in the present study were located at these sites, our data highlighted that these changes did not significantly affect the effectiveness of RAT kits. The results were concordant with our previous studies that RT-PCR was at least 100-fold more sensitive than the RAT kits against different SARS-CoV-2 strains.¹⁴⁻¹⁸

The main objective of the current study is to estimate if the commercially available RAT kits are capable of detecting Omicron BA.2. It is not our primary concern to give RAD kits an accurate ranking. In addition to the two serial tenfold dilution points, it is ideal to perform 1:2 and 1:5 dilution points between them, especially C_t values around 25-30 which is close to the LOD of RAT kits. Furthermore, each dilution point should be repeated two or more times. This evaluation performed overlapped with the highest surge of COVID-19 cases in Hong Kong.⁶ As extra specimens and RAD kits were required, despite limited manpower and resources, we determined that 10-fold serial dilutions and one duplicate would be the most appropriate study design to use. On the basis of this study design, RAD kits would perform similarly when the LODs exhibited within tenfold difference.

There were several limitations in the current study. First, we only used one Omicron BA.2 virus to assess analytical sensitivity. However, the sample we used was representative of the BA.2 lineage. Among the BA.2 sequences available from GISAID, >95% of them had five mutations/changes, P13L, 31-33 deletion of ERS, R203K, G204R, and S413R. Our sequencing results showed that the strain used in the present study also shared these mutations/changes, other mutations/changes were not found. Although rare, mutations of the N gene have been reported to result in false-negative RAT results even when viral loads of samples are high. These sporadic strains were found to have T205I, D399N, and P279Q mutations. The highest sensitivity loss

was 1000-fold for these variants.^{19,20} At the time of revising this report on April 2022, several descendent lineages, BA.4 and BA.5 were noted.²¹ The BA.4 is of particular concern since it had an additional mutation in the N gene, P151S has not been commonly found in BA.1, BA.2, BA.3, and BA.5.²² Data and analysis on this variant are limited with a high level of uncertainty. Further studies are required to assess the effect of this mutation on the performance of RAT kits and if this variant will become the dominating lineage in the future. Second, we only measured the LOD of RAT kits. As a consequence, results should be interpreted with caution, in particular, should not be used to infer clinical sensitivity. Although analytical sensitivity does not reflect clinical sensitivity, our previous studies showed that analytical sensitivity correlated well with clinical sensitivity.¹³⁻¹⁵ In addition, all of the RAT kits evaluated in the present study could detect concentration C_t 23.13, which was in line with the recent review of summarized 24 studies worldwide.²³ This review concluded that RAT kits were sensitive for detecting samples of $C_t \leq 25$. The LOD results enable us to assess RAT kits quickly when numerous kits are evaluated. Our results, therefore, showed that the RAT kits used in this study may be used for the first-line screening of Omicron BA.2 cases. Third, the sample we used was combined nasopharyngeal and throat swabs, which was not the sample type, nasal swab, recommended by the kit inserts. In our previous evaluations, the sensitivity of RAT kits is viral load-dependent. Since our study focuses on analytical sensitivity, each dilution point was thoroughly mixed and homogeneous, sample type was not a variable in our study. Finally, we did not test for specificity for RAT kits. However, this issue was not the major concern in view of the currently evaluated RAT kits.²⁴

5 | CONCLUSION

The evaluation results of different RAT kits are important to help us to implement the test appropriately. Due to the emergence of different SARS-CoV-2 variants as well as the latest developed

RAT kits, the performance of RAT kits should be regularly monitored so that guidance can be provided to different clinical settings.

AUTHOR CONTRIBUTIONS

Gannon C. K. Mak: Conceptualization, methodology, validation, and investigation; writing—original draft; and writing—review and editing. **Stephen S. Y. Lau:** Validation and investigation. **Kitty K. Y. Wong:** Validation and investigation. **Chi. -Shan. Lau:** Resources and supervision. **Ken H. L. Ng:** Supervision. **Edman T. K. Lam:** Supervision. **Rickjason C. W. Chan:** Supervision.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to the current study as the data are comprehensively described throughout this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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