Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology 144 (2021) 104994

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Comparison of analytical sensitivity of the eight rapid antigen detection kits for detecting SARS-CoV-2 virus

Gannon C.K. Mak *, Stephen S.Y. Lau, Kitty K.Y. Wong, Nancy L.S. Chow, C.S. Lau, Edman T. K. Lam, Rickjason C.W. Chan, Dominic N.C. Tsang

Microbiology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region, China

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Rapid antigen detection
RT-PCR

1. Introduction

Although rapid antigen detection (RAD) kits are inferior to RT-PCR for detection of SARS-CoV-2 in terms of sensitivity, these kits satisfy the demand for an early diagnosis due to the ease of use and rapid results [1].

The SARS-CoV-2 is continuously evolving, different strains with different phenotypic and genotypic changes were identified. Different systems were proposed to call these SARS-CoV-2 strains such as 'variant' [2, 3]. We have evaluated different RAD kits and found that the performance of RAD kits varied between different brands [4–6]. However, there is very limited data on parallel comparison of the performance of RAD kits against different SARS-CoV-2 strains. The aim of this evaluation is to assess analytical sensitivity of RAD kits by means of limit of detection (LOD) using serial dilution of clinical specimens.

2. Methods

2.1. Respiratory specimens

Two clinical specimens were selected for this evaluation. Throat saliva was obtained from a Hong Kong COVID-19 patient (hCoV-19/Hong Kong/VB20097960/2020) collected on 9 Jul 2020. Another throat saliva was obtained from a COVID-19 patient returned from UK (hCoV-19/Hong Kong/CM20000424/2020) collected on 7 Dec 2020. It was a 501Y.V1 variant as confirmed by the presence of key non-synonymous substitutions in spike (S) gene.

To note: at the time of starting the evaluation, our lab was capable of detecting two variants 501Y.V1 [7] and 501Y.V2 [8]. Since there is no universal nomenclature system to name SARS-CoV-2 variants, the names 501Y.V1 and 501Y.V2 are used throughout this publication. 501Y.V1 and 501Y.V2 were first identified by the intensive surveillance system targeting returning travelers to Hong Kong [9, 10]. They are different from the SARS-CoV-2 strains detected in Hong Kong. Our S gene surveillance [11] showed that these two variants harbored ≥10 non-synonymous substitutions, while the Hong Kong cases sequenced between Jan 2020 and Dec 2020 harbored only a maximum of four non-synonymous substitutions [12]. Since the viral load of clinical specimens for 501Y.V1 were high, serial dilution could be performed and hence, 501Y.V1 was selected in the present study.

2.2. RAD kits for SARS-CoV-2 detection

A total of eight kits were selected in the present study, all of them were commercially available in Hong Kong. Six of them were supplied by local suppliers during the period from October 2020 to November 2020 which have not been evaluated by us before. The remaining two were previously evaluated [5, 6]. These two kits were also under the 'WHO Emergency Use Listing for In vitro diagnostics Detecting SARS-CoV-2' [13]. All of the kits were procured by the Public Health Laboratory Services Branch of center for Health Protection, Department of Health (Hong Kong Special Administrative Region) for this

* Corresponding author at: 9/F, Public Health Laboratory center, 382 Nam Cheong Street, Shek Kip Mei, Kowloon, Hong Kong Special Administrative Region, China

E-mail address: so_phls10@dh.gov.hk (G.C.K. Mak).

https://doi.org/10.1016/j.jcv.2021.104994
Received 8 January 2021; Received in revised form 9 September 2021; Accepted 29 September 2021
Available online 2 October 2021

Table 1
Comparison of RT-PCR and rapid antigen detection kits for the limit of detection against two SARS-COV-2 strains.

Dilution	hCoV-19/Hong Kong/VB20097960/2020[a]									hCoV-19/Hong Kong/CM20000424/2020[b]								
	RT-PCR[d]	Rapid antigen detection kits[c]								RT-PCR[d]	Rapid antigen detection kits[c]							
		1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8
original	16.13	ND	ND	ND	ND	ND	ND	ND	ND	16.20	ND	ND	ND	ND	ND	ND	ND	ND
10^{-1}	20.95	+	+	+	+	+	+	+	+	21.29	+	+	+	+	+	+	+	+
$10^{-1.3}$	22.16	+	+	+	+	+	+	+	+	22.17	+	NEG	+	+	+	+	+	+
$10^{-1.7}$	23.26	+	+	+	+	+	+	+	+	22.78	+	NEG	+	+	NEG	+	+	+
10^{-2}	24.38	+	NEG	+	+	+	+	+	+	24.39	NEG	NEG	+	+	NEG	NEG	+	+
$10^{-2.3}$	25.76	+	NEG	+	+	+	NEG	+	+	25.50	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
$10^{-2.7}$	27.18	+	NEG	+	+	NEG	NEG	+	+	26.64	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
10^{-3}	28.25	+	NEG	+	+	NEG	NEG	+	+	28.08	ND	ND	ND	ND	ND	ND	ND	ND
$10^{-3.3}$	28.82	NEG	NEG	+	NEG	NEG	NEG	+	+	28.86	ND	ND	ND	ND	ND	ND	ND	ND
$10^{-3.7}$	30.27	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	29.84	ND	ND	ND	ND	ND	ND	ND	ND
10^{-4}	31.55	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	31.66	ND	ND	ND	ND	ND	ND	ND	ND
$10^{-4.3}$	32.66	ND	ND	ND	ND	ND	ND	ND	ND	32.00	ND	ND	ND	ND	ND	ND	ND	ND
$10^{-4.7}$	34.27	ND	ND	ND	ND	ND	ND	ND	ND	34.55	ND	ND	ND	ND	ND	ND	ND	ND
10^{-5}	35.79	ND	ND	ND	ND	ND	ND	ND	ND	34.79	ND	ND	ND	ND	ND	ND	ND	ND
10^{-6}	NEG	ND	ND	ND	ND	ND	ND	ND	ND	NEG	ND	ND	ND	ND	ND	ND	ND	ND

Abbreviations: + means positive; NEG means negative; ND means not done.
[a] Throat saliva collected from a Hong Kong COVID-19 patient on 9Jul2020.
[b] Throat saliva collected from a COVID-19 patient returned from UK on 7Dec2020, the 501Y.V1 variant.
[c] 1 = Aegle; 2=ARISTA; 3 = Biosynex; 4 = INDICAID; 5 = LumiraTEK; 6 = Wondfo; 7 = Standard Q; 8 = Panbio.
[d] RT-PCR were tested twice with identical results. The Ct values shown were the mean of both runs.

evaluation.

All of the kits employ a lateral flow test format to detect viral antigen by the immobilized coated SARS-CoV-2 antibody on the device. The test results can be interpreted without a reader. The details for each kit was summarized in Table S1. For the ease of communication, 'Aegle', 'ARISTA', 'Biosynex', 'INDICAID', 'LumiraTEK', 'Wondfo', 'Standard Q', 'Panbio' stand for these kits.

2.3. Limit of detection

There were two dilution sets. Each set was a serial tenfold dilution from 10^{-1} to 10^{-8}. Within each dilution from 10^{-1} to 10^{-5}, further dilution of 1:2 and 1:5 were also performed for each point.

In order to unify the input volume, a fixed amount of throat salvia was used. Regarding the input volume, we previously evaluated two input volumes, 100 μL and 350 μL. The result bands were more intense when using 350 μL specimen volume [6] and was selected for accessing analytical sensitivity in the present study. Each test was carried out first by mixing the 350 μL specimen volume with the kit's extraction buffer/diluent. Then the subsequent procedures were carried out according to manufacturer's instructions. The intended use for RAD kits is for swab samples. Although throat saliva is not intended use for RAD kits, we previously found that viral load in sample is the main factor affecting the performance of RAD kits. If the sample is not viscous in nature, sample type is not the significant issue in our evaluation [4–6].

Virus concentrations in each dilution were estimated from cycle threshold (Ct) value as described [4].

The LOD of the RAD kits and RT-PCR for the two dilution sets were performed in two different days. Within the same set of dilution, both RAD kits and RT-PCR were performed on the same day without freeze and thaw cycle. For RAD test, one replicate was performed for each dilution point due to limited quantity of kits procured. For RT-PCR, duplicates were performed for each dilution point.

3. Results

The LOD results were summarized in Table 1.

When RAD kits were tested against the local strain, five of them shared similar sensitivity, the LOD were between 10^{-3} and $10^{-3.3}$ (Ct values 28.25 and 28.82). The remaining three kits were less sensitive that those five kits, the LOD were between $10^{-1.7}$ and $10^{-2.3}$ (Ct values ranging from 23.26 to 25.76).

When the RAD kits were tested against the variant strain, decrease in sensitivity was observed for all of the kits. The LOD for four of them were 10^{-2} (Ct value 24.39), the other four were 10^{-1} and $10^{-1.7}$ (Ct values ranging from 21.29 to 22.78).

4. Discussion

The purpose of this study is to evaluate the analytical sensitivity of different commercially available RAD kits for diagnosing SARS-CoV-2 infection including the variant strain. Our study demonstrated that three of them shared similar analytical sensitivity to the two kits recommended by WHO when tested against the local strain. This local strain represented the peak of COVID-19 cases detected in Hong Kong between July-August 2020 which was characterized by the 12F amino acid in the spike protein. It was only detected in this specific time interval and then disappeared [12].

The sensitivity of RAD kits was decreased when testing against the 501Y.V1 variant. However, the decrease in sensitivity was not significant, with a maximum of 10 fold difference. RAD kits detected lowest concentrations at Ct 24 and 28 for the 501Y.V1 variant and the local strain respectively. We have ruled out the effect of diluent used to perform the serial dilution when tested with the two WHO recommended kits (Table S2). In addition, the decreased in sensitivity was reproducible when testing with another 501Y.V1 strain from the pooled nasopharyngeal and throat swab which was obtained from a COVID-19 patient (hCoV-19/Hong Kong/VM21000371/2021) returned from France collected on 3 January 2021 [14]. Similar decrease in sensitivity was also found when tested with the two WHO recommended kits (Table S3). However, the impact on clinical sensitivity remains unknown.

The limitations of this study include the fact that we only assess analytical sensitivity of RAD kits and the LOD results do not directly reflect clinical sensitivity. However, our previous studies showed that LOD results were correlate well with clinical sensitivity [4–6]. The LOD approach provides a quick screening method when numerous kits are considered for an extended evaluation. Other limitations include the fact that we did not test for specificity for RAD kits. However, data on specificity for the currently available RAD kits were consistently reported to be high [1]. On the other hand, due to the limited availability of SARS-CoV-2 variants, it was not sure if the decreased in sensitivity would appeal in other SARS-CoV-2 strains. Unlike RT-PCR which detects nucleic acids, antigen tests depend on the amount of viral proteins

expressed in the sample.

In conclusion, understanding the performance of RAD kits can guide us to implement the test appropriately. Irrespective of different SARS-CoV-2 strains, our study showed that RAD kits were good at detecting high viral load samples and was in-line with other head to head RAD kits' comparisons [15, 16]. It is expected that many RAD kits for diagnosing SARS-CoV-2 infection are emerged in this exploding field. Some RAD kits can be available over the counter and can be self-tested at home. In our setting, around 1/3 of the SARS-CoV-2 positive specimens were above Ct 28 (Table S4). There is no evidence that COVID-19 cases above Ct 28 are not infectious [17]. Inappropriate use of RAD kits is deleterious to our COVID-19 control strategy and hence RAD kits should be used with cautions.

Declaration of Competing Interest

None.

Acknowledgement

We thank our colleagues Peter K.C. Cheng, Alan K.L. Tsang and Peter C.W. Yip for technical assistance of identifying SARS-CoV-2 variants.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2021.104994.

References

[1] WHO. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance, 11 September 2020. Available at: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (Accessed 26 March 2021).

[2] WHO. Weekly epidemiological update –23 February 2021. Available at: https://www.who.int/publications/m/item/weekly-epidemiological-update—23-february-2021 (Accessed 26 March 2021).

[3] E. Callaway, A bloody mess': confusion reigns over naming of new COVID variants, Nature 589 (7842) (2021) 339, https://doi.org/10.1038/d41586-021-00097-w. JanPMID: 33452513.

[4] G.C. Mak, P.K. Cheng, S.S. Lau, K.K. Wong, C.S. Lau, E.T. Lam, R.C. Chan, D. N. Tsang, Evaluation of rapid antigen test for detection of SARS-CoV-2 virus, J. Clin. Virol (2020), https://doi.org/10.1016/j.jcv.2020.104500. Aug;129: 104500.Epub 2020 Jun 8. PMID: 32585619; PMCID: PMC7278630.

[5] G.C. Mak, S.S. Lau, K.K. Wong, N.L. Chow, C.S. Lau, E.T. Lam, R.C. Chan, D. N. Tsang, Analytical sensitivity and clinical sensitivity of the three rapid antigen detection kits for detection of SARS-CoV-2 virus, J. Clin. Virol 133 (2020), 104684, https://doi.org/10.1016/j.jcv.2020.104684. DecEpub 2020 Oct 29. PMID: 33176236; PMCID: PMC7598567.

[6] G.C.K. Mak, S.S.Y. Lau, K.K.Y. Wong, N.L.S. Chow, C.S. Lau, E.T.K. Lam, R.C. W. Chan, D.N.C. Tsang, Evaluation of rapid antigen detection kit from the WHO emergency use list for detecting SARS-CoV-2, J. Clin. Virol 134 (2020), 104712, https://doi.org/10.1016/j.jcv.2020.104712. Dec 4Epub ahead of print. PMID: 33338894; PMCID: PMC7716730.

[7] Public Health England. Investigation of novel SARS-COV-2 variant variant of concern 202012/01. Version 1, release date 21/122020. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/959438/Technical_Briefing_VOC_SH_NJL2_SH2.pdf (Accessed 26 March 2021).

[8] H. Tegally, E. Wilkinson, M. Giovanetti, et al., Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa, bioRxivorg (2020), https://doi.org/10.1101/2020.12.21.20248640.

[9] Hong Kong Free Press. Covid-19: gov't barring Hongkongers returning from UK, despite earlier stance on returnees from China, 24 December 2020. Available at: https://hongkongfp.com/2020/12/24/covid-19-govt-barring-hongkongers-returning-from-uk-despite-earlier-stance-on-returnees-from-china/(Accessed 26 March 2021).

[10] Radio Television Hong Kong. HK sees first mutant covid strain from South Africa, 31 December 2020. Available at: https://news.rthk.hk/rthk/en/component/k2/1568042-20201231.htm (26 March 2021).

[11] G.C.K. Mak, A.W.L. Lau, A.M.Y. Chan, D.Y.W. Chan, D.N.C. Tsang, The D614G substitution in the S gene and clinical information for patients with COVID-19 detected in Hong Kong, J. Clin. Virol 130 (2020), 104550, https://doi.org/10.1016/j.jcv.2020.104550. SepEpub 2020 Jul 24. PMID: 32763812; PMCID: PMC7378481.

[12] G.C.K. Mak, A.W.L. Lau, A.M.Y. Chan, E.T.K. Lam, R.C.W. Chan, D.N.C. Tsang, The surveillance of spike protein for patients with COVID-19 detected in Hong Kong in 2020, J. Med. Virol 93 (9) (2021) 5644–5647, https://doi.org/10.1002/jmv.27063. SepEpub 2021 May 24. PMID: 33951208; PMCID: PMC8242547.

[13] WHO. WHO emergency use listing for in vitro diagnostics (IVDs) detecting SARS-CoV-2 (last updated: 2 October 2020). Available at: https://www.who.int/diagnostics_laboratory/201002_eul_sars_cov2_product_list.pdf?ua=1 (Accessed 26 March 2021).

[14] Asia Times. HK reports 15 mutant virus cases in two weeks, 5 January 2021. Available at: https://asiatimes.com/2021/01/hk-reports-15-mutant-virus-cases-in-two-weeks/(Accessed 26 March 2021).

[15] J. Favresse, C. Gillot, M. Oliveira, J. Cadrobbi, M. Elsen, C. Eucher, K. Laffineur, C. Rosseels, S. Van Eeckhoudt, J.B. Nicolas, L. Morimont, J.M. Dogné, J. Douxfils, Head-to-head comparison of rapid and automated antigen detection tests for the diagnosis of SARS-CoV-2 infection, J. Clin. Med 10 (2) (2021) 265, https://doi.org/10.3390/jcm10020265. Jan 13PMID: 33450853; PMCID: PMC7828347.

[16] N. Eshghifar, A. Busheri, R. Shrestha, S. Beqaj, Evaluation of analytical performance of seven rapid antigen detection kits for detection of SARS-CoV-2 virus, Int. J. Gen. Med 14 (2021) 435–440, https://doi.org/10.2147/IJGM.S297762. Feb 12PMID: 33603450; PMCID: PMC7886288.

[17] CDC. Testing, isolation, and quarantine for persons who have recovered from previous SARS-CoV-2 infection. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/faq.html (Accessed 26 March 2021).