Virology

Evaluation of automated antigen detection test for detection of SARS-CoV-2

Gannon C.K. Mak*, Stephen S.Y. Lau, Kitty K.Y. Wong, Nancy L.S. Chow, C.S. Lau, Ken H.L. Ng, 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, Hong Kong, China

A B S T R A C T

RT-PCR is the gold standard to detect SARS-CoV-2, however, its capacity is limited. We evaluated an automated antigen detection (AAD) test, Elecsys SARS-CoV-2 Antigen (Roche, Germany), for detecting SARS-CoV-2. We compared the limit of detection (LOD) between AAD test, rapid antigen detection (RAD) test; SARS-CoV-2 Rapid Antigen Test (SD Biosensor, Korea), and in-house RT-PCR test. LOD results showed that the AAD test was 100 fold more sensitive than the RAD test, while the sensitivity of the AAD test was comparable to the RT-PCR test. The AAD test detected between 85.7% and 88.6% of RT-PCR-positive specimens collected from COVID-19 patients, false negative results were observed for specimens with Ct values >30. Although clinical sensitivity for the AAD test was not superior or comparable to the RT-PCR test in the present study, the AAD test may be an alternative to RT-PCR test in terms of turn-around time and throughput.

© 2021 Elsevier Inc. All rights reserved.

1. Introduction

Successful COVID-19 control could only be achieved by widespread testing, contacts tracing and cases isolation. RT-PCR is the gold standard and the most widely used method to detect SARS-CoV-2 (Carter et al., 2020; Vandenberg et al., 2021). For rapid diagnosis of SARS-CoV-2 infection, rapid antigen detection (RAD) tests for qualitative determination of SARS-CoV-2 antigen are available. RAD tests are fast and can be performed by healthcare professional without intensive training and specialized instrument. However, application of these tests are limited to the high viral load samples (Mak et al., 2020a; Mak et al., 2020b; Mak et al., 2020c). With the limited capacity of RT-PCR tests, it is not known whether the sudden increased of COVID-19 cases will lead to the increased demand for SARS-CoV-2 diagnosis. Alternatives have to be sought for other tests which shared similar turn-around time, throughput and even clinical performance with RT-PCR.

The purpose of this evaluation was to evaluate an automated antigen detection (AAD) test in detecting SARS-CoV-2. The first part of the study was to assess the limit of detection (LOD) between AAD, RAD and RT-PCR tests. The second part was to evaluate the performance of AAD test in detecting SARS-CoV-2 in different types of respiratory specimens.

2. Methods

2.1. Respiratory isolates

2.1.1. SARS-CoV-2

The dilution set of the SARS-CoV-2 culture isolate (strain hCoV-19/Hong Kong/VM20001097/2020, the first SARS-CoV-2 case detected in Hong Kong) was used to determine LOD between different tests (Mak et al., 2020a). The sensitivity of different tests can be obtained by measuring the lowest concentration of the culture isolate. RT-PCR was the gold standard among different tests, the viral load of different dilution points were estimated by the cycle threshold (Ct) values.

To prepare the dilution set, 1:100 dilution was performed for the stock of the culture supernatant. Then, serial tenfold dilution was performed to obtain a dilution set from 10^{-2} to 10^{-8}. Each dilution point was aliquoted and stored at -70°C until further testing.

2.1.2. Non-SARS-CoV-2 respiratory virus isolates

To evaluate the cross-reactivity of the AAD test, 13 non-SARS-CoV-2 respiratory virus isolates were tested. They were influenza A (H1), influenza A(H3), influenza B, adenovirus, coronavirus type OC43, coronavirus type 229E, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3, parainfluenza virus type 4, respiratory syncytial virus, rhinovirus and enterovirus. Except OC43 and 229E, all of the isolates were obtained from our routine

culture of respiratory specimens received from other hospitals and clinics. OC43 and 229E were kindly provided by the Department of Microbiology, University of Hong Kong.

2.2. Respiratory specimens

From January 6, 2021 to March 12, 2021, respiratory specimens from COVID-19 patients collected by the Public Health Laboratory Services Branch in Hong Kong were retrieved for this evaluation. All of the specimens were confirmed with SARS-CoV-2 infection by RT-PCR as described (Mak et al., 2020a).

The Ct values used to classify specimens as 'high viral load', 'medium viral load' and 'low viral load' were identical to previous studies, they were <18.57, 18.57-28.67 and >28.67 respectively (Mak et al., 2020b; Mak et al., 2020c).

The types of specimens selected were nasopharyngeal swab (NPS) and combined nasopharyngeal swab and throat swab (NPS & TS). A total of 35 specimens each for NPS and NPS & TS were selected. Since we were out of specimens, there were some minor deviation for the ratio of viral load distribution, 1:2:1, for 'high viral load', 'medium load' and 'low viral load' specimens as described (Mak et al., 2020b).

An additional of 20 NPS specimens collected between September and October 2009, prior to the introduction of SARS-CoV-2, were also included to evaluate the cross-reactivity of the AAD test. These specimens were previously tested positive for influenza viruses.

2.3. Antigen detection kits used

2.3.1. RAD kit

The Roche 'SARS-CoV-2 Rapid Antigen Test' (SD Biosensor, Korea) kit was selected in the present study to compare LOD between different tests. This kit was under the 'WHO Emergency Use Listing for In vitro diagnostics (IVDs) Detecting SARS-CoV-2' (2021). Another name of this kit was Standard Q COVID-19 Ag. The procedures were carried out according to manufacturer's instructions.

2.3.2. AAD kit

The 'Elecsys SARS-CoV-2 Antigen' (Roche, Germany) kit was chosen in the present study. The intended use for this kit was for NPS and oropharyngeal swab specimens, 30uL of sample volume was required to transfer to the reaction container. The principles was based on electrochemiluminescence and an analyzer was required. The cobas e 801 analyzer was used in the present study, it has been installed in our setting for performing routine serology tests. There were no hands on procedures except transferring samples to the reaction containers (including the dead volume of the reaction container) and loading them to the analyzer. After starting the test, the results were available in 18 minutes. This instrument allows for continuous loading of samples and a maximum of 300 samples can be loaded. Positive and negative results were automatically outputted from the analyzer and were shown as 'Reactive' or 'Non-reactive'. All of the calculation steps were done by the instrument, the results were calculated by using Cutoff Index (COI). COI ≥1.0 and <1.0 were for 'Reactive' and 'Non-reactive' results respectively.

2.4. Sample preparation

All samples tested for the AAD test were inactivated by using 'SARS-CoV-2 Extraction Solution C' (Roche, Germany). To inactivate SARS-CoV-2 samples, the SARS-CoV-2 Extraction Solution C was mixed with samples in 1:10 ratio. Due to the limited quantity for each specimen, 150 uL of each sample was used. It means that 15 uL SARS-CoV-2 Extraction Solution C was mixed with 150 uL samples in the present study. After standing at room temperature for 2 minutes, the inactivated samples were transferred to reaction containers and proceeded for the AAD test.

Table 1

Comparison of RT-PCR, rapid antigen detection (RAD) and automated antigen detection (AAD) tests for the limit of detection of SARS-CoV-2.

Dilution[b]	Test results[a]				
	Inactivated samples			Un-treated samples	
	RT-PCR[c]	RAD	AAD	RT-PCR[c]	RAD
10^{-2}	18.78	POS	POS	19.61	POS
10^{-3}	22.86	POS	POS	24.19	POS
10^{-4}	27.47	POS	POS	27.96	POS
10^{-5}	29.74	NEG	POS	31.60	NEG
10^{-6}	36.07	NEG	POS	34.90	NEG
10^{-7}	NEG	ND	NEG	NEG	ND
10^{-8}	NEG	ND	NEG	NEG	ND

[a] ND = not done; POS = positive; NEG = negative.
[b] Serial tenfold dilution of the SARS-CoV-2 culture isolate, hCoV-19/Hong Kong/VM20001097/2020 (case 1 of the Hong Kong patient).
[c] RT-PCR were tested twice in the same run with identical results. The Ct values shown were the mean of both runs.

In order to see if there were any effects of 'SARS-CoV-2 Extraction Solution C' on RAD and RT-PCR tests, LOD was determined using inactivated samples and untreated samples.

2.5. RT-PCR

The in-house developed RT-PCR test was used to detect the presence of SARS-CoV-2 nucleic acid in all samples as described previously (Mak et al., 2020a). It targets the large polyprotein ORF1ab of SARS-CoV-2. Viral RNA amount in respiratory specimens were estimated from cycle threshold (Ct) value.

3. Results

The LOD of the RAD test was 100 fold less sensitive than SARS-CoV-2 RT-PCR test which was concordant to our previous study. We previously compared the SARS-CoV-2 Rapid Antigen Test (SD Biosensor, Korea) kit with the in-house RT-PCR test, the SARS-CoV-2 Rapid Antigen Test was 100 fold less sensitive than the in-house RT-PCR test (Mak et al., 2020b). The LOD of the AAD test was 10^{-6} which was comparable to RT-PCR test. When comparing the inactivated samples and un-treated samples, the LOD for RAD and RT-PCR tests remained the same for these two group of samples (Table 1).

We also evaluated variability between runs for the AAD test. LOD was performed in two different days, and the results were the same. The COI values were shown in Table S1.

In the cross-reactivity test using virus isolates and NPS collected prior to the introduction of SARS-CoV-2, all were tested negative by the AAD test.

Of the 70 specimens tested, the AAD test can detect all high viral load and medium viral load specimens (100%, N = 57) but low sensitivity for low viral load specimens (28.6%−33.3 %) (Table 2). Review of the COI values showed that some values were varied from the viral load of the specimens estimated by Ct values, however, COI values were correlated with Ct values in general (Table S2).

4. Discussion

In this study, we determined the performance characteristics of the Elecsys SARS-CoV-2 Antigen kit for detecting SARS-CoV-2 respiratory specimens and compared the results with RAD test while RT-PCR test was selected as the gold standard. To the best of our knowledge, there is no peer-reviewed study of evaluating this kit.

The methodology used in the present study was same as before for evaluating RAD kits (Mak et al., 2020a; Mak et al., 2020b; Mak et al., 2020c). Confounding factors such as types of specimens processed and method to quantity the viral load were limited. Our

Table 2
Performance characteristics of the automated antigen detection (AAD) test for the presence of SARS-CoV-2 in 70 respiratory specimens.

| Specimen type[a] | Specimens used for the AAD test and the results | | | | |
| | Ct value | | No. of specimens | | Sensitivity |
	Mean	Range	Tested	positive	
NPS					
High	16.56	13.65-18.44	13	13	100
Medium	22.85	18.60-27.62	15	15	100
Low	32.84	28.81-34.96	7	2	28.6
All	22.51	13.65-34.96	35	30	85.7
NPS & TS					
High	16.73	14.90-18.36	12	12	100
Medium	21.57	18.58-24.67	17	17	100
Low	32.30	29.46-35.40	6	2	33.3
All	21.75	14.90-35.40	35	31	88.6

[a] 'High', means specimens with Ct values <18.57 of SARS-CoV-2 RT-PCR; 'Medium', Ct values between 18.57 and 28.67; 'Low', Ct values >28.67.

data indicated that the AAD and RT-PCR tests shared similar LOD results. In terms of clinical sensitivity, the AAD test is better than the RAD test (Table S3). The AAD test showed 100% sensitivity for detected specimens with Ct values <30. For low viral load specimens, although declined sensitivity was found, the AAD test can detect specimens up to 31.69.

The AAD test evaluated in the present study was a high throughput test. Hands on procedures were mainly the sample preparation steps and the samples loading steps to the analyzer. To handle around 100 specimens by two laboratory technologists, we estimated that around 1.5 hours were required from samples preparation to results interpretation. For RT-PCR, it normally requires 3 hours to process 100 specimens from RNA extraction, PCR reagent dispensing to thermal cycling. We have to say that the AAD test is not suitable for low-complexity laboratories. An analyzer is required to install in the laboratory and intensive training is needed. AAD test is different to the RAD test while RAD test is a point of care test. On the other hand, the cobas e 801 analyzer is mainly for testing serum specimens in our setting. There are rigid sample requirements for this analyzer and is not suitable for viscous specimens such as throat saliva and sputum. Swab samples were less affected as they were immersed in the viral transport media and were homogenized. In addition, bubbles generated especially during the sample inactivating steps will be rejected by the analyzer. Extra procedures were required to ensure the reaction containers were free of bubbles. For example, a disposable dropper was required to aspirate the bubbles on the top.

The limitations of this study include the fact that we employed a small sample size to evaluate the AAD test. In addition, it remains unclear whether the AAD test is applicable to laboratories like us for receiving throat saliva as majority specimen type for confirmation. However, our results were in-line with other AAD systems to detect SARS-CoV-2. These studies showed that other AAD systems were capable of detecting low viral load SARS-CoV-2 specimens or having higher sensitivity than the RAD tests (Favresse et al., 2021; Gili et al., 2021; Wang et al., 2021). The VITROS test (Ortho Clinical Diagnostics, USA) showed a sensitivity of 100% when clinical samples of Ct values ≤33 were tested. On the contrary RAD tests were mostly effective to identify clinical samples with Ct values ≤25 (Favresse et al., 2021). The S-PLEX test (MesoScale Diagnostics, USA) was capable of detecting 95.4% of clinical samples after excluding samples with Ct values >35 (Wang et al., 2021). The Lumipulse G test (Fujirebio, Japan) also

shared similar performance when clinical samples were tested with Ct cutoff of 35 (Gili et al., 2021).

In summary, the gold standard to detect SARS-CoV-2 remains RT-PCR test in terms of sensitivity and specificity. The AAD test is a method of choice and serves as adjunct to RT-PCR test when there is an upsurge of specimens or when large number high risk group of individuals are required for testing.

Declaration of competing interest

The authors declare that they have not received any funding for this study and no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contributions

Gannon CK Mak: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing. Stephen SY Lau: Validation, Investigation. Kitty KY Wong: Validation, Investigation. Nancy LS Chow: Validation, Investigation. CS Lau: Resources, Supervision. Ken HL Ng: Supervision. Edman TK Lam: Supervision. Rickjason CW Chan: Supervision, Writing - original draft, Writing - review & editing. Dominic NC Tsang: Supervision, Writing - original draft, Writing - review & editing.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2021.115490.

References

Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay techniques and test development for COVID-19 diagnosis. ACS Cent Sci 2020;6(5):591–605. doi: 10.1021/acscentsci.0c00501.

Favresse J, Gillot C, Oliveira M, Cadrobbi J, Elsen M, Eucher C, et al. Head-to-head comparison of rapid and automated antigen detection tests for the diagnosis of SARS-CoV-2 infection. J Clin Med 2021;10(2):265. doi: 10.3390/jcm10020265.

Gili A, Paggi R, Russo C, Cenci E, Pietrella D, Graziani A, et al. Evaluation of Lumipulse® G SARS-CoV-2 antigen assay automated test for detecting SARS-CoV-2 nucleocapsid protein (NP) in nasopharyngeal swabs for community and population screening. Int J Infect Dis 2021;105:391–6. doi: 10.1016/j.ijid.2021.02.098.

Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol 2020a;129:104500. doi: 10.1016/j.jcv.2020.104500.

Mak GC, Lau SS, Wong KK, Chow NL, Lau CS, Lam ET, et al. Analytical sensitivity and clinical sensitivity of the three rapid antigen detection kits for detection of SARS-CoV-2 virus. J Clin Virol 2020b;133:104684. doi: 10.1016/j.jcv.2020.104684.

Mak GCK, Lau SSY, Wong KKY, Chow NLS, Lau CS, Lam ETK, et al. Evaluation of rapid antigen detection kit from the WHO Emergency Use List for detecting SARS-CoV-2. J Clin Virol 2020c;134:104712. doi: 10.1016/j.jcv.2020.104712.

Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol 2021;19(3):171–83. doi: 10.1038/s41579-020-00461-z.

Wang H, Hogan CA, Verghese M, Solis D, Sibai M, Huang C, et al. Ultra-sensitive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen detection for the diagnosis of coronavirus disease 2019 (COVID-19) in upper respiratory samples. Clin Infect Dis 2021:ciab063. doi: 10.1093/cid/ciab063.

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 April 29, 2021.