SARS-CoV-2 Detection on Artificially Contaminated Surfaces by Rapid Antigen Test

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ABSTRACT

Objective: Evaluation of an antigen-based rapid test for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on artificially contaminated objects in comparison with a real-time reverse transcription-polymerase chain reaction (RT-qPCR) standard method.

Materials and Methods: Artificial surface contamination with inactivated SARS-CoV-2 was tested on ten different objects comprising fruits and common materials. Three contamination levels with virus titers of 10^3, 10^4, and 10^5 pfu/100 µl were studied. Each object was spiked with 200 µl of virus suspension, samples were then collected by swabbing and evaluated by rapid antigen test and RT-qPCR. Additionally, 3- and 5-day contamination with SARS-CoV-2 at 10^5 pfu/100 µl was tested for some materials.

Results: The detection rate obtained by the rapid antigen test with 10^3, 10^4, and 10^5 pfu/100 µl of SARS-CoV-2 was 10%, 90%, and 90%, respectively for the tested objects. RT-qPCR showed a detection rate of 100% at all virus titers. Furthermore, both rapid antigen test and RT-qPCR were able to detect the 3- and 5-day extended contamination with SARS-CoV-2.

Conclusion: The collected data suggests that the evaluated rapid antigen test is suitable for detection of SARS-CoV-2 adhered to non-human samples as a screening method. This simple method can reduce costs and turnaround time when compared to a standard molecular assay. It may be applied to enhance safety policies for COVID-19 prevention in public health and international export-businesses.

Keywords: SARS-CoV-2; COVID-19; Rapid antigen test; RT-qPCR, screening method; surface contamination (Siriraj Med J 2023; 75: 7-12)

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the pandemic coronavirus disease 2019 (COVID-19). Transmission of infectious SARS-CoV-2 to the human respiratory tract occurs through two major pathways: by aerosols/droplets in direct person-to-person contact and via exposure to contaminated fomites in indirect contact. Viable SARS-CoV-2 has been shown to survive on different surfaces for days or weeks depending on temperature, relative humidity, and light.[1]

High safety standards are a must in the food industry, including in food processing and distribution to maintain consumer trust and confidence in its products. However, infected food workers, either unaware of hygiene guidelines or not following them, might contaminate food during processing and packaging by touching it with contaminated hands or via infectious droplets released when talking, coughing, or sneezing.[2] SARS-CoV-2 contamination of food products and packaging materials can lead to serious economic loss in food export businesses. For example, China, known for its strict COVID-19 policy,

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temporarily banned durian from Thailand due to several positive SARS-CoV-2 detection results during random testing.[3] Although the contact with SARS-CoV-2 adhered on food, including fruits and vegetables, or food packaging materials is highly unlikely to cause COVID-19, such contaminations must always be tracked, particularly in the actual context where the virus is spreading in the countries.[4]

Real-time reverse transcription polymerase chain reaction (RT-qPCR) is recognized as the gold standard method for the detection of SARS-CoV-2 in clinical and non-clinical samples. However, it is limited by a long turnaround time due to nucleic acid extraction and amplification and requires trained staff, expensive instruments, and a laboratory setting with adequate biosafety. These resources are not always available in all countries and in this case a rapid antigen test might be an alternative to RT-qPCR. While it is less sensitive, it is faster, easier to perform, more affordable and allows for decentralized testing at field areas.[5]

At the present time, data on the use of rapid antigen tests to detect SARS-CoV-2 in food or environmental samples are limited. Thus, this study aimed to evaluate the performance of an antigen-based rapid test for detection of SARS-CoV-2 on artificially surface-contaminated objects in comparison with a RT-qPCR standard method.

MATERIALS AND METHODS

Inactivated SARS-CoV-2 virus preparation

An inactivated clinical isolate of SARS-CoV-2/01/human/Jan2020/Thailand was used in this study. It represented the original Wuhan strain isolated from a confirmed COVID-19 patient at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand.

The inactivated virus was prepared by two methods, heating and UV-C radiation. Stock SARS-CoV-2 virus of 10^6 pfu/ml was divided into two sets for incubation at 65°C, 15 min, and for exposure by UV-C for 15 min. Subsequently, the virus was inoculated onto Vero E6 cells to confirm the complete inactivation of the virus by absence of cytopathic effects (CPE).

All processes involving inactivated SARS-CoV-2 were performed under Enhanced BSL-2 (BSL-2+) in accordance with the biosafety guidelines. The project was approved by the Thammasat University Institutional Biosafety Committee (101/2564).

Artificial-surface contamination and sample collection

Serial dilutions of 10^3, 10^4, and 10^5 pfu/100 µl were prepared from the stocks of heat- and UV-C-inactivated SARS-CoV-2. Samples of pooled inactivated virus at each dilution were prepared by combining 100 µl each of heat- and UV-C-inactivated SARS-CoV-2. Ten different objects comprising common fruits and packaging materials were selected for analysis. They were durian, rambutan, orange, apple, leather, parcel box, fruit foam net, foam box, foil, and plastic.

Inoculation and swab processes were performed by different persons. Pooled inactivated virus of each dilution was randomly spiked, by making tiny drops with pipette like droplets from sneezing, onto the entire surface of each object and the objects were then completely dried at room temperature. The objects were collected by randomly swabbing without knowledge of previous inoculation site at an area of 100 to 225 cm^2 or entire area for smaller ones at day 0, 3 and 5. Two swabs were used for SARS-CoV-2 detection by rapid antigen test and RT-qPCR.

SARS-CoV-2 testing

Nucleocapsid (NP) protein antigen of SARS-CoV-2 was detected by a Rapid Surface Ag 2019-nCov Kit (Prognosis Biotech, Larissa, Greece). Briefly, the collected swab was placed in extraction buffer for 30 seconds and was then discarded. Afterwards, a test strip was immersed into the extraction buffer for 10 min. Detection of SARS-CoV-2 resulted in visible colored bands at both Test (T) and Control (C) lines. As shown in the test manual, cross-reactivity with 4 different human coronavirus strains is not found, and the limit of detection (LOD) is 2.5 ng/ml of NP or 5.75 x 10^3 $TCID_{50}$/ml of inactivated SARS-CoV-2.[6]

Collected swabs for RT-qPCR assay were kept in HiViral™ transport medium (HiViral™ Transport Kit, HiMedia, Mumbai, India). Swabs were vortexed and 200 µl of HiViral™ transport medium was used to extract RNA by using a PureLink viral RNA/DNA mini kit (Cat no. 12280050, Invitrogen, USA) according to the manufacturer's instructions. The concentration of the purified RNA was measured as ng/µl and the RNA was kept at −80°C before RT-qPCR detection. Following the manufacturer's instructions and interpretations, SARS-CoV-2 RNA targeting ORF1ab, N, and E genes was detected by an ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit (Cat no. 3103010069, 3DMed, Germany).

Positive (SARS-CoV-2) and negative (human coronavirus strain OC43) controls were used to validate results in all experiments.

Statistical analysis

Descriptive analysis as mean, standard deviation (SD), detection rate (%) was performed and compared between rapid antigen test and RT-qPCR at each viral dilution.

RESULTS

Detection of SARS-CoV-2 by rapid antigen test and RT-qPCR on artificially contaminated objects was compared and the results obtained on the day of inoculation and sample collection (day 0) are shown in Table 1 and Fig 1. RT-qPCR, the gold standard method, had a higher sensitivity than the rapid antigen test and detected SARS-CoV-2 contamination on all objects at all virus dilutions. The detection rate obtained with the rapid antigen test was 10%, 90% and 90% at 10^3, 10^4 and 10^5 pfu/100 µl, respectively (Fig 1). The sensitivity of the rapid test was poor at the lowest virus titer but was much improved at 10^4 and 10^5 pfu/100 µl. Likewise, the intensity of the detected T-band seemed to depend on the virus titer (Fig 2). However, we observed that the type of material affected the detection. Detection of SARS-CoV-2 contamination was most difficult for both methods on the parcel box made from paper. Indeed, even contamination with virus titer at 10^4 and 10^5 pfu/100 µl showed negative results when detected by the rapid test. Although it could be detected by RT-qPCR, the Ct values of all target genes were shifted over ten cycles (Table 1). Additionally, plastic was the only object out of the ten spiked objects that could be detected by the rapid test at 10^3 pfu/100 µl SARS-CoV-2.

Next, we investigated the detection rate after the artificially contaminated objects were left for 3 and 5 days. All objects spiked with 10^5 pfu/100 µl SARS-CoV-2 could be detected by rapid test and RT-qPCR after 3 and 5 days (Table 2). The results were consistent with the same day testing (day 0).

Further comparison of RT-qPCR and rapid antigen test showed the latter to have a limit for detection of SARS-CoV-2 NP when the Ct values (mean±SD) of RT-qPCR targeting the ORF1ab, N, and E genes were in the range of 30.77±3.74, 27.02± 3.64, 26.54±9.72, respectively (Table 1).

DISCUSSION

This study used pooled heat and UV-C inactivated SARS-CoV-2 to contaminate ten different materials. Heat-inactivation at 65°C for 15 min will denature viral proteins but not the genomic RNA, while UV-C-inactivation for 15 min has a deleterious effect on the RNA but not on the viral structure.[7] Thus, the pooled inactivated SARS-CoV-2 used in this study allowed parallel application of the two detection methods, i.e., antigen-based rapid test and nucleic acid-based RT-qPCR and minimized the risk of false negative results.

The used rapid chromatographic immunoassay intended for qualitative detection had a lower sensitivity in SARS-CoV-2 detection in comparison to the gold standard method RT-qPCR. Our data showed that the limit of detection of the rapid antigen test was at 10^4 pfu/100 µl. At this amount of virus RT-qPCR showed average Ct values for ORF1ab, N, and E genes, across the analyzed samples in the range of 30.77±3.74, 27.02±3.64, 26.54±9.72, respectively.

However, the results of the rapid test showed that detection sensitivity depended on the kind of investigated material. SARS-CoV-2 NP could be still detected at 10^3 pfu/100 µl on plastic, whereas it could not be detected at a titer as high as 10^5 pfu/100 µl on other materials like parcel box. Interestingly, Ct values from SARS-CoV-2 detection by RT-qPCR showed the highest value at all virus titers on parcel box. Previous research supports these findings.[8-9] Most of the enveloped viruses like SARS-CoV-1 or influenza virus were found to survive and persist in stable form longer on plastic and stainless steel (1–7 days) than on paper and tissue (3–8 h).[9-11] SARS-CoV-2 was found to be inactivated much faster on paper than on plastic. No virus could be detected after 3 hours of being inoculated on paper.[8,10] Corpet hypothesized that dryness would inactivate SARS-CoV-2 like found on water absorbent porous materials.[10] Since an enveloped virus has a lipid bilayer membrane that needs water on both sides to maintain an intact structure dryness might lead to oxidation of lipids and Maillard reactions of proteins.[10] While smooth and waterproof materials would protect the virus by keeping the moisture from micro-droplets of water on the surface.[12] This would explain the stability of SARS-CoV-2 on non-absorbent materials, including durian, leather, and plastic on which it could be detected after many days by both, rapid test and RT-qPCR.

Taken together, our pilot study on artificially contaminated objects suggests that the used rapid antigen test would be a valuable method for screening of different materials. In comparison to RT-qPCR it is easier to perform, would cost less, save time, and is suitable for a large number of samples. Its application may enhance safety policies in public health and international export-businesses. However, the limitations in this study were using only artificial samples under controlled conditions and no testing with control group of inoculation with non-infected fluid on samples that might develop interpretation bias on an antigen-based rapid test. Thus, these concerns should be considered for future study. Real-world samples should be done with and always in comparison with a gold standard RT-qPCR assay.

TABLE 1. Comparison of SARS-CoV-2 detection results on artificially contaminated objects by rapid antigen test and RT-qPCR on same day testing (day 0).

Samples	Cycle threshold (Ct) value (Interpret result) of RT-qPCR detection[a]					Rapid antigen test result
	ORF1ab	N gene	E gene	Internal control	Conclusion result[b]	
A. Virus titer at 10^5 pfu/100 µl						
SARS-CoV-2[c]	11.94 (+)	12.27 (+)	9.38 (+)	34.03 (+)	+	+
HCOV-OC43[d]	> 40 (-)	> 40 (-)	> 40 (-)	> 40 (-)	-	-
Durian	27.1 (+)	23.47 (+)	25.83 (+)	> 40 (-)	+	+
Rambutan	28.84 (+)	26.49 (+)	27 (+)	> 40 (-)	+	Weak +
Orange	26.55 (+)	23.05 (+)	25.12 (+)	> 40 (-)	+	+
Apple	24.14 (+)	21.52 (+)	22.6 (+)	> 40 (-)	+	+
Leather	21.46 (+)	20.27 (+)	20.07 (+)	> 40 (-)	+	+
Parcel box	37.56 (+)	34.2 (+)	35.62 (+)	39.71 (+)	+	-
Fruit foam net	25.44 (+)	22.8 (+)	24.33 (+)	38.89 (+)	+	+
Foam box	25.49 (+)	23.11 (+)	24.12 (+)	39.79 (+)	+	+
Foil	24.95 (+)	21.96 (+)	25.33 (+)	> 40 (-)	+	+
Plastic	23.57 (+)	20.61 (+)	23.12 (+)	> 40 (-)	+	+
Mean±SD (Positive-Ct)	**26.51±4.37**	**23.75±4.07**	**25.31±4.10**	**11.84±19.06**		
B. Virus titer at 10^4 pfu/100 µl						
SARS-CoV-2	16.35 (+)	16.05 (+)	15.16 (+)	37.19 (+)	+	+
HCOV-OC43	> 40 (-)	> 40 (-)	> 40 (-)	> 40 (-)	-	-
Durian	31.57 (+)	28.57 (+)	29.93 (+)	38.15 (+)	+	Weak +
Rambutan	32.63 (+)	30.32 (+)	31.54 (+)	> 40 (-)	+	Weak +
Orange	31.17 (+)	26.45 (+)	29.16 (+)	38.64 (+)	+	Weak +
Apple	36.06 (+)	28.69 (+)	> 40 (-)	> 40 (-)	+	Weak +
Leather	27.36 (+)	22.63 (+)	27.24 (+)	37.43 (+)	+	Weak +
Parcel box	36.95 (+)	34.45 (+)	35.63 (+)	> 40 (-)	+	-
Fruit foam net	30.13 (+)	26.71 (+)	30.2 (+)	38.19 (+)	+	Weak +
Foam box	29.46 (+)	25.62 (+)	28.96 (+)	35.99 (+)	+	Weak +
Foil	26.06 (+)	23.33 (+)	26.48 (+)	> 40 (-)	+	Weak +
Plastic	26.32 (+)	23.42 (+)	26.27 (+)	> 40 (-)	+	+
Mean±SD (Positive-Ct)	**30.77±3.74**	**27.02±3.64**	**26.54±9.72**	**18.84±19.87**		
C. Virus titer at 10^3 pfu/100 µl						
SARS-CoV-2	19.89 (+)	19.30 (+)	18.44 (+)	> 40 (-)	+	+
HCOV-OC43	> 40 (-)	> 40 (-)	> 40 (-)	> 40 (-)	-	-
Durian	36.99 (+)	32.73 (+)	35.11 (+)	38.32 (+)	+	-
Rambutan	31.79 (+)	29.71 (+)	31.21 (+)	> 40 (-)	+	-
Orange	34.39 (+)	30.10 (+)	33.49 (+)	> 40 (-)	+	-
Apple	28.46 (+)	25.27 (+)	27.67 (+)	> 40 (-)	+	-
Leather	32.39 (+)	28.17 (+)	32.75 (+)	> 40 (-)	+	-
Parcel box	37.48 (+)	36.47 (+)	36.27 (+)	> 40 (-)	+	-
Fruit foam net	35.49 (+)	29.06 (+)	> 40 (-)	> 40 (-)	+	-
Foam box	33.45 (+)	30.03 (+)	33.04 (+)	37.40 (+)	+	-
Foil	31.97 (+)	28.31 (+)	32.31 (+)	37.58 (+)	+	-
Plastic	32.01 (+)	28.97 (+)	32.11 (+)	> 40 (-)	+	Weak +
Mean±SD (Positive-Ct)	**33.44±2.73**	**29.88±2.98**	**29.40±10.58**	**11.33±18.24**		

[a] "+" when Ct value ≤ 40, and "-" when Ct value ≥ 40.
[b] Conclusion results were interpreted following the manufacturer's instruction. In brief, positive when at least 2/3 of SARS-CoV-2 specific RNA targets were detected without relying on internal control detection.
[c] Positive control from inactivated SARS-CoV-2.
[d] Negative control from inactivated human coronavirus strain OC43 (HCOV-OC43).

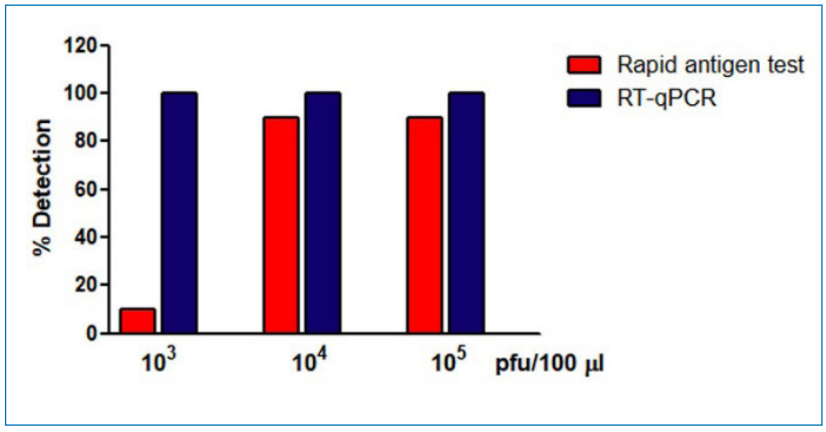

Fig 1. Comparison of detection rate (%) between rapid antigen test and RT-qPCR.
The relative number of positive results per total samples at each tested virus titer is shown as percentage of detection.

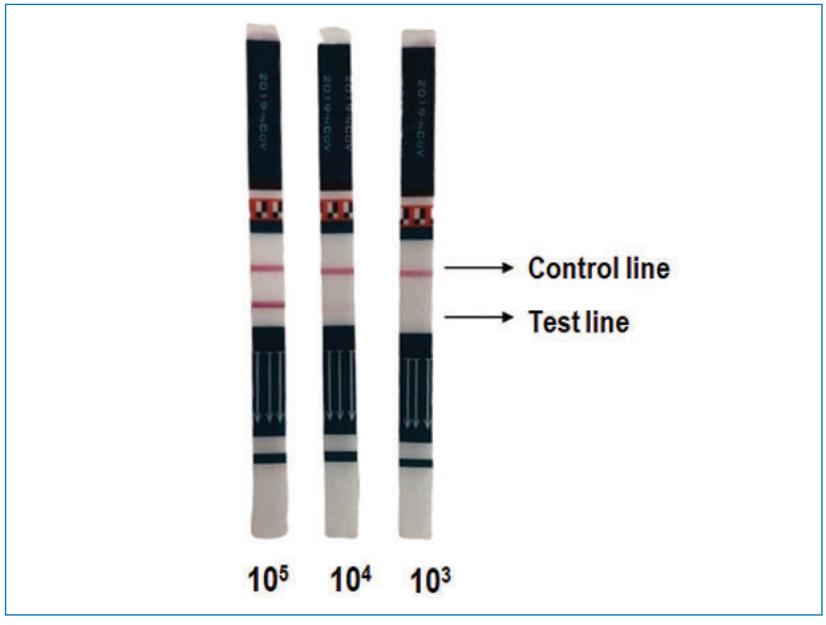

Fig 2. Test line intensity of rapid antigen test.
From left to right, 10^5, 10^4, and 10^3 pfu/100 µl SARS-CoV-2 virus titers were evaluated by rapid antigen tests. The observed test line intensity depended on the virus titer. The results were interpreted as positive, weak positive, and negative, respectively.

TABLE 2. Comparison of SARS-CoV-2 detection results on artificially contaminated objects by rapid antigen test and RT-qPCR after 3 and 5 days of inoculation.

Samples	Cycle threshold (Ct) value (Interpret result) of RT-qPCR detection[a]					Rapid antigen test result
	ORF1ab	N gene	E gene	Internal control	Conclusion result[b]	
Day 3-Virus titer at 10^5 pfu/100 µl						
Durian	21.91 (+)	20.88 (+)	21.14 (+)	> 40 (-)	+	+
Leather	30.08 (+)	26.27 (+)	31.09 (+)	> 40 (-)	+	+
Plastic	24.60 (+)	21.11 (+)	25.54 (+)	33.21 (+)	+	+
Day 5-Virus titer at 10^5 pfu/100 µl						
Durian	24.77 (+)	23.27 (+)	23.73 (+)	> 40 (-)	+	+
Leather	28.97 (+)	24.96 (+)	30.08 (+)	> 40 (-)	+	+
Plastic	23.88 (+)	20.53 (+)	24.84 (+)	39.01 (+)	+	+

[a] "+" when Ct value ≤ 40, and "-" when Ct value ≥ 40.
[b] Conclusion results were interpreted following the manufacturer's instruction. In brief, positive when at least 2/3 of SARS-CoV-2 specific RNA targets were detected without relying on internal control detection.

CONCLUSION

This study suggests the rapid antigen test as a first screening assay to identify SARS-CoV-2 contamination on various material types. It would reduce the demand for the expensive and time-consuming RT-qPCR assay in non-clinical samples.

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