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COVID-19 Antibody Rapid Testing Kit (Ab-RTK): An Assessment of its Specificity, Sensitivity, Precision and Accuracy and Diagnostic Correlation to Radiologic Tests Results

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Abstract: **COVID-19 caused by SARS-CoV-2 has grown into a pandemic in less than two months. The importance of having a specific and accurate laboratory testing cannot be under stated. Although diagnostic testing by RT-PCR is both accurate and specific and considered to be the gold standard, there is a need of a test that is fast, reliable and readily available. Serological testing of IgG and IgM antibodies in patient's blood has been proposed to be a rapid diagnostic tool to COVID-19 diagnosis. We evaluated a commercially available COVID-19 Antibody-Rapid Testing Kit (Ab-RTK), Innovita, developed for rapid (within 15 minutes) detection of SARS-CoV-2-specific IgG and IgM using 12 PCR-confirmed COVID-19 cases, 20 PCR-confirmed negative cases,10 healthy controls and 10 non-COVID cases. An overall accuracy of 90.6% with 75.0% sensitivity for true positive cases and 100% sensitivity for true negative cases were achieved. The assay specificities were shown to be 58.3% and 75% respectively for IgM and IgG. The IgG antibodies remain to be detected >14 days after a negative rtPCR testing of a previously rtPCR positive subject. Formation of antibodies appear to be directly correlated with a positive/ significant chest radiologic examination result.**

Keywords: **COVID-19, SARS-CoV-2, Ab-RTK, IgG/IgM Antibody Rapid Testing.**

1. BACKGROUND

COVID-19 (Coronavirus Disease 2019), caused by the novel SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2), spread rapidly and developed into a global pandemic within three months from its initial detection in December 2019 in Wuhan, Hubei Province, China [1]. The most common symptoms are fever, tiredness, and dry cough [2]. Some patients may have body aches and pains, nasal congestion, runny nose, sore throat and diarrhea. These symptoms are usually mild and begin gradually [3-4]. One out of every 6 people who gets COVID-19 becomes seriously ill and experience difficulty of breathing [2]. In the Philippines, the first reported case in February 2020 manifested as pneumonia. As of April 23, 2020, the Department of Health reported more than 6,000 confirmed cases and >400 human deaths [5]. Community transmission have been documented. Though most of the confirmed COVID-19 cases are symptomatic, the virus has been detected in completely asymptomatic individuals. Due to the variable manifestations, clinical diagnosis becomes problematic. Confirmation of COVID-19 positivity requires detection of SARS-CoV-2 nucleic acid by reverse-transcriptase polymerase chain reaction (rtPCR) [6-7]. There are limited rtPCR testing facilities in the country and not all suspects nor contacts of COVID-19 cases are tested. It is presumed that the total number of actual

COVID-19 cases in the country is much higher than the number of confirmed ones. On average, RTPCR results from this region are released after 5-10 days after specimen collection. Thus, COVID-19 Rapid Antibody Test (RAT) has suggested to be used as an adjunct tool for the diagnosis in correlation with clinical manifestations and while waiting for rtPCR results. We evaluated a commercially available antibody rapid test kit (Ab-RTK) , the Innovita 2019-nCoV antibody test (Colloidal Gold) ,Tangshan Biological Technology Co., Ltd. – No. 699 Juxin Street, High-Tech Industrial Development Zone, Qian'an 064400, Hebei, China, developed for detection of SARS-CoV-2-specific antibodies. We validated its output versus known rtPCR positive and rtPCR negative individuals. The main objective is to assess the Ab-RTK in terms of specificity, sensitivity, precision and accuracy. We also correlated the antibody detection with chest radiologic examination results.

2. MATERIALS AND METHODS

Serum samples

Venous blood samples collected using a gold SST tube with serum separator from 12 PCR-confirmed positive COVID-19 patients, 20 RT-PCR-confirmed negative patients for COVID-19, 10 healthy asymptomatic individuals, and 10 symptomatic-non-COVID patients, were included in the study. Nine out of 12 samples from confirmed positive patient were collected >21 days after onset of symptom while 3 out of 12 were collected < 21 days after symptom onset. All blood samples from the 20 PCR confirmed negative patients were collected between 15-26 days after onset of symptoms. Samples from 10 healthy asymptomatic individuals were included and serve as the negative control while 10 samples from symptomatic non-COVID patients were used to determine specificity of the Ab-RTK. Results of Chest X-ray and chest Ct-scan were correlated with antibody production.

Innovita 2019-nCoV antibody test kit (COLLOIDAL GOLD)

The test was run according to the manufacturer's instructions (COVID-19 IgG/IgM Rapid Test Cassette (whole blood/serum/plasma), Lot: 20200401, (Tangshan) Biological Technology Co., Ltd. – No. 699 Juxin Street, High-Tech Industrial Development Zone, Qian'an 064400, Hebei, China) [8]. Briefly, 10 µl of serum were added to the test kit well, followed by 2 drops of the buffer provided in the kit. The results were read within 15 minutes by the naked eye. Only tests in which the control line changed its color were regarded as valid. If a line was observed for IgM and/or IgG, the test was considered positive. The intensity of the color was not judged. At least 2 trained medical technologists concurred before declaration of final result. Positive on either IgG or IgM on the RAT is considered positive result.

3. RESULTS

Reactivity of Rapid Test Kit IgG and IgM in Healthy Control Samples

None of the ten (10) serum samples from asymptomatic healthy individuals were tested IgM and IgG positive in the assay. It shows 100% (10/10) accuracy.

Table 1: Comparisons of Antibody results for PCR-positive COVID-19 cases, PCR-confirmed negative cases, and Non-COVID Symptomatic Cases

Antibody Testing Result	PCR-confirmed Positive Cases	PCR-confirmed Negative Cases	Non-COVID Cases
IgG/IgM Positive	9/12 (75.0%)	0/20 (0%)	0/10 (0%)
IgG/ IgM Negative	3/12 (25.0%)	20/20 (100%)	10/10 (100%)

Reactivity of Rapid Test Kit IgG and IgM in PCR-confirmed positive cases, PCR-confirmed negative cases, and Non-COVID Symptomatic cases

Innovita antibody rapid test kit detected antibodies in 75.0% in 12 PCR-confirmed cases. Three of 12 samples were false negative. On the other hand, no antibodies were detected in PCR-confirmed negative cases for 100% sensitivity.

Table 2: Antibody Test kit Sensitivity in Terms of the Number Days after Onset of Symptoms Using a Sample from PCR-confirmed Cases

	Days After Onset of Symptoms	
Antibody Class	< 21 days	>21 days
IgM Positive	1/3 (33.3%)	6/9 (66.7%)
IgG Positive	0/3 (0%)	9/9 (100%)

Reactivity of Rapid Test Kit IgG and IgM in PCR-confirmed positive cases Based on the number of days after onset of symptoms

Innovita Ab-RTK detected IgM antibodies in 1 of 3 rtPCR positive samples collected <21 days after symptom onset while a 66.7% of rtPCR positive samples collected >21 days showed positive for IgM antibodies. Ab-RTK did not detect IgG antibodies from a sample collected <21 days after symptom onset but a 100% IgG detection from a sample collected >21 days after symptom onset.

Table 3: Presence of Antibody from Samples collected 14 days after Negative rtPCR test

Antibody Class	Number of positive cases sera 1	Number of positive cases sera 2
IgM	7/9	0/9
IgG	9/9	9/9

Reactivity of IgG and IgM in Samples collected 14 days after a Negative PCR Testing

Paired sera from nine patients positive for rtPCR collected initially showed reactivity to either/both IgM and IgG. Fourteen days later, all nine patients retested negative for rtPCR, and tested 100% for IgG only on Ab-RTK.

Assay sensitivity, specificity and accuracy

Table 4: Tabular representation of the Sensitivity, Specificity, Precision, and Accuracy and the Negative Predictive Value of the Rapid Antibody Test Kit

		RT-PCR RESULT (Gold Standard)		
		Confirmed Positive (12)	Confirmed Negative (20)	
RAPID ANTIBODY TESTING (RAT) RESULT	RAT Positive (9)	True Positive (9)	False Positive (0)	**Positive Predictive Value (Precision)** (100%)
	RAT Negative (3)	False Negative (3)	True Negative (20)	**Negative Predictive Value** (100%)
		Sensitivity (75%)	**Specificity** (100%)	**Accuracy** (91%)

Table 5: Correlates of IgG and IgM Formation with Radiologic Examination Results

PCR Confirmed Positive Case	With SIGNIFICANT Radiologic Exam result	Without SIGNIFICANT Radiologic Exam result
IgG/IgM Positive	9/9	0/8
IgG/IgM Negative	0/9	8/8

Correlation of chest X-ray & Ct-Scan with the presence of IgG and IgM by Ab-RTK

Nine of the 12 PCR confirmed positive cases with IgG/IgM positivity on Ab-RTK showed positive findings of pneumonitis and pneumonia in X-ray and or ground glass opacity in CT-scan while three serologically negative but PCR confirmed positive had normal or insignificant results in chest X-ray or chest CT scan.

4. DISCUSSION

We evaluated a commercially available antibody rapid test kit, Innovita, for the detection of SARS-CoV-2-specific IgM and IgG. Samples from PCR-confirmed positive COVID-19 cases, obtained during disease or convalescence, were used as 'true positive'. (Table 4). Of the 12 true positive samples, three did not show antibody detection by Ab-RTK thus considered as false negative. These cases may have not developed antibodies yet to COVID 19 virus or may truly be negative. These three false negative cases, were asymptomatic and did not develop changes on their chest X-rays and CT scans contrary to the other 9 rtPCR positive individuals with positive SARS-CoV2 antibodies, who showed significant lung changes in CXR and chest CT scan. Whether this findings imply immune reactivity as a necessary event to develop pathological lung changes and symptoms remain to be proven.

For a more ideal evaluation of the rapid test kit sensitivity, an immunologic test gold standard for SARS-CoV-2-specific antibodies should have been used. However, such assay is not available to date. Our study is also limited by our access to rtPCR and its results.

For the PCR-confirmed negative cases and the samples from asymptomatic healthy individual, Innovita Ab-RTK has shown 100% specificity with 0% false negative results. Our results conform to the manufacturer's declared specificity evaluated on 14 PCR-negative samples and was found to be 100% for both IgM and IgG. The calculated sensitivity our assays with Ab-RTK Innovita is 75%. The results by Li et al. indicated an overall testing sensitivity of 88.7% and 90.6% specificity [9]. Our results showed a lower sensitivity (75%) for both IgG and IgM but a higher specificity of 100%.

In-vitro testing manufacturer's report for Ab-RTK Innovita is 87.3% sensitivity and 100% specificity [8].

Seroconversions for COVID 19 occurs between 7 and 12 days after the onset of symptoms [10-11]. A larger detailed kinetics of the antibody responses is necessary. Our study showed detectable IgM and IgG at day 21 after symptom onset. We were not able to document immunoglobulin detection at the early phase of the disease, especially for IgM antibodies. Only one sample taken less than 21 days showed a positive IgM result. Our cohort showed persistence of IgG, but not of IgM, after 14 days of having had a negative rtPCR detection for patients previously testing positive on rtPCR. The impact of early or late seroconversion on the case severity and disease progression, nor a suggestion of fitness and disease freedom is not yet known. The persistence of IgG antibodies after a negative rt PCR may implicate seroconversion and may be useful to evaluate individuals for plasma donation.

The 100% accuracy for true negative cases of this rapid test kit may be a presumptive evidence of COVID-19 negative infection. A larger cohort is necessary to validate this assumption. A negative Ab-RTK , maybe useful to give an added layer of confidence for health care workers entertaining diagnosis other than COVID 19, in close correlation with clinical and radiologic studies for initial, but not conclusive assessment of patients while waiting for the more definitive rtPCR results.

In general, this study showed a satisfactory performance of the antibody rapid test kit Innovita (AB-RTK), given the limitations of this study. The researchers recommend that this assay be compared to other validated serological tests and be validated against a larger number of rtPCR samples. For now, we find less indications in using this test for clinical diagnosis. Ab-RTK should be treated and used only as an adjunct for diagnosis. Careful interpretation against clinical and radiologic data, might contribute to making informed decisions in triage and decision for patient isolation. Ab-RTK positive patients can be labelled as highly probable COVID cases while awaiting for PCR testing. Likewise, if symptomatic and Ab-RTK positive, patients most likely will show significant radiologic chest findings. The high negative predictive value indicates that the rapid test will be useful for detecting past infections and possible indicator of immunity development, which may be crucial for restoring social functions and reducing the stigma on previously positive rtPCR patients.

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Disclosure statement

The authors declare no conflicts of interest.

REFERENCES

[1] Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., ... & Xing, X. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England Journal of Medicine*. https://www.nejm.org/doi/full/10.1056/NEJMOa2001316

[2] World Health Organization COVID-19 Worldwide Bulletin, April 13, 2020. [Crossref], [Google Scholar]

[3] Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061–1069. doi:10.1001/jama.2020.1585

[4] Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cheng, Z. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, *395*(10223), 497-506. https://doi.org/10.1016/S0140-6736(20)30183-5

[5] Department of Health Website. . [Crossref] doh.gov.ph/2019-nCOV

[6] Department of Health Circular No. 2020-0160 dated March 31, 2020. Guidance on the Use of COVID-19 Rapid Antibody-based Test Kits

[7] The Philippine Society for Microbiology and Infectious Diseases (PSMID) Guidelines on the Use and Interpretation of rapid antibody-based testing kits. Available from https://bit.ly./PSMID-COVID-19.

[8] Innovita Product Insert, Biological Technology Co., Ltd. – No. 699 Juxin Street, High-Tech Industrial Development Zone, Qian'an 064400, Hebei, China,

[9] Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol [Internet]. 2020 Feb 27. Cited 2020 Apr 4;jmv:25727. Available from:: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25727 [Crossref], [Google Scholar]

[10] Zhang J, Liu J, Li N, et al. Serological detection of 2019-nCoV respond to the epidemic: A useful complement to nucleic acid testing. medRxiv. 2020;10:20030916. [Google Scholar]

[11] Zhao R, Li M, Song H, et al. Serological diagnostic kit of SARS-CoV-2 antibodies using CHO-expressed full-length SARS-CoV-2 S1 proteins. medRxiv. 2020;27:20042184. [Google Scholar]

[12] Maria Infantino et al. Diagnostic accuracy of an automated chemiluminescent immunoassay for anti-SARS-CoV-2 IgM and IgG antibodies: an Italian experience. https://onlinelibrary.wiley.com/doi/10.1002/jmv.25932