Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Evaluation of Immunochromatographic Assay-Ag and Ab for Rapid Detection of SARS-Corona Virus-2 against Real-Time Polymerase Chain Reaction

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Received: 20 Feb 2025; **Accepted:** 05 Apr 2025; **Published:** 17 Apr 2025

Citation: Mohamed Hussien Kambal, Abu Baker Altom Ahmed, Nadir Abuzeid, et al. PEvaluation of Immunochromatographic Assay-Ag and Ab for Rapid Detection of SARS-Corona Virus-2 against Real-Time Polymerase Chain Reaction. Microbiol Infec Dis. 2025; 9(2): 1-5.

ABSTRACT

Since 2019, SARS-Corona virus-2 has been confirmed as causing acute respiratory illness worldwide with significant morbidity and mortality rates. There is an urgent need for fast, reliable diagnostic techniques for the purpose of control measures. The present study aimed to evaluate the sensitivity and specificity of rapid immunochromatography tests for detecting SARS-CoV-2 antigens and antibodies (ICT-Ag/Ab) against the approved polymerase chain reaction (PCR) technique. One hundred and two nasopharyngeal swabs were collected from symptomatic patients. An additional twenty-five specimens were received as negative controls. Real-time PCR (RT-PCR) for the detection of nuclear and envelope genes was carried out. ICTs for the detection of Ags and Abs were performed for the two groups and compared with the molecular method. Low sensitivity was detected for both ICTs, Ag/Ab as 59.8% and 32.4%, respectively. The calculated specificity was also low for ICT-Ag (48%) and ICT-Ab (48%) against the RT-PCR technique. Among observed symptoms, a significant association (p-value 0.004) was determined for throat pain with ICT-Ag within the RT-PCR positive group. No significant difference was determined for ICT-Ab among observed symptoms within the RT-PCR positive group (p-value > 0.05). The study revealed moderate sensitivity and low specificity for ICT/Ag and decreased values of both sensitivity and specificity for ICT/Ab compared with RT-PCR.

Keywords

Coronavirus, Immunochromatography, RT-PCR, Ag.

Introduction

The first cases of Coronavirus disease 2019 (COVID-19) were reported from Wuhan, China, in December 2019, and it progressed rapidly. On 30 January, WHO declared the new disease, then declared it a pandemic on 11 March. Subsequent molecular studies confirmed that the origin of this transmissible pneumonia was the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, which causes the new COVID-19 disease [1]. Coronaviruses are large-sized (100–160 nm), spherical, enveloped, non-segmented, positive-sense RNA viruses, known to be broadly distributed in humans and other mammals [2]. The majority of the infected persons are asymptomatic or experience mild to moderate respiratory illness and recover without requiring special treatment; however, they may transmit the virus to other susceptible individuals. It can cause a variety of acute and chronic

diseases. Common signs of a person infected with coronaviruses include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe acute cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [2]. The clinical diagnosis of COVID-19 is slightly complicated, and cases in which the infection spreads from asymptomatic infected individuals are numerous. The laboratory diagnosis of SARS-CoV-2 infection mostly relies on three categories of testing reverse transcription polymerase chain reaction (rRT-PCR), antigens detect SARS-CoV-2, and the third type looks for antibodies in the human body (IgM, IgG). In Japan, the nucleic acid amplification tests (NAAT) are mainly used for definitive diagnosis [3,4].

In the early phase of the pandemic, the sensitivity of PCR was reported to be approximately 70% [5], and this low sensitivity was due to the use of oropharyngeal swabs. In addition, the peak viral shedding of COVID-19 occurs from the day before the onset of illness to about 2-3 days after the onset of illness [6], and the results of RT-qPCR tend to vary in the later stages of illness [7,8]. The sensitivity of the test in the early stages of the disease is often high, which is approximately 90% according to a meta-analysis [9]. Despite the lower sensitivity of the antigen test compared with the PCR method [10], it is recommended that rapid antigen tests be used in Japan during the period when the viral load is likely to be high [11]. Nasopharyngeal or nasal swabs are commonly used as specimens, but saliva specimens are not warranted due to poor diagnostic performance [12,13].

The antibody tests are many methods, but immunochromatographic antibody testing should not be used because of possible cross-reactivity with other antibodies and low correlation with neutralizing antibodies [14]. Cassaniti compared the results of rapid serological tests with those of nasopharyngeal swabs in 30 healthy subjects, 30 RT-PCR-SARS-CoV-2-positive subjects, and 50 subjects admitted to the emergency department with fever and respiratory syndrome: all 30 healthy subjects were negative by rapid IgM/IgG testing; of the 30 positive subjects, 19 (63.3%) showed clear positivity for both IgM and IgG antibodies, five (16.7%) weak positivity for both, one (3.3%) and isolated IgM positivity. Interestingly, among the 50 patients admitted to the emergency department, 38 tested COVID-19-positive by RT-PCR; of these 38, only seven patients showed a positive rapid test with a sensitivity of 18.4% and a specificity of 91.7%, NPV 26.2%, PPV 87.5% [15].

Methods

Study design

This is a case-control analytical study that was carried out in Alribat Teaching Hospital (RTH). Study subjects were patients with signs of upper respiratory tract infection. Subjects were admitted to the emergency room at different times after the onset of symptoms.

Ethical consideration

The study was approved by the Ethical Review Committee of Alribat Teaching Hospital.

Study Samples

Consecutive nasopharyngeal swabs and blood specimens were collected from 102 symptomatic patients RT-PCR positive) and an additional 25 samples as negative control (RT-PCR negative) for COVID-19 antigen and antibody (IgM/IgG) detection.

Molecular Analysis

The Xpert Xpress SARS-CoV-2 test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens intended for the qualitative detection of nucleic acid gene (O gene) and envelope gene (E gene), which containing primers and probes and internal controls used in RT-PCR [16].

Immunoassay

Rapid Chromatographic Immunoassay (ICT) for the qualitative detection of specific antigen to SARS-CoV-2 present in human nasopharynx. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region, and mouse monoclonal anti-chicken IgY antibody SARS-CoV-2 antibody is coated on the test line on the surface of the nitrocellulose membrane [17]. ICT for qualitative detection of anti-coronavirus antibodies IgG/IgM in human serum was carried out. The IgG/IgM antibody in the sample combined with the recombinant new coronavirus antigen labeled with the colloidal gold on the binding pad formed complex [18].

Statistical Analysis

The specificity, sensitivity, and predictive values of ICTs were calculated at a 95% confidence interval using GraphPad Prism software.

Results

All (127) subjects enrolled in this study underwent a SARS-CoV-2 RT-PCR, rapid Ag test, and rapid IgM/IgG antibody test. Of all, 74 (58.3%) were males and 53(41.7%) were females.

Table 1: Distribution of gender among case and control.

Gender	Frequency	%
Male	74	58.3
Female	53	41.7
Total	127	100.0

Distribution of Symptoms among RT-PCR-positive and Negative Groups

The most frequently observed symptom among the RT-PCR-positive group was fever (52%) followed by cough (45.1%), shortness of breath (35.3%), and throat pain (33.3%). Among the RT-PCR negative control group, the same loom was observed as fever was most frequent (48%) followed by cough (44%), shortness of breath (28%), and throat pain (16%) (Figure 1, 2).

Sensitivity and Specificity Result

Low sensitivity was detected for both ICTs (Ag/Ab) as 59.8% and 32.4%, respectively. In the same line, the calculated specificity was low for ICT Ag (48%) and ICT Ab (48%) against the RT-PCR technique. Table 2 & 3.

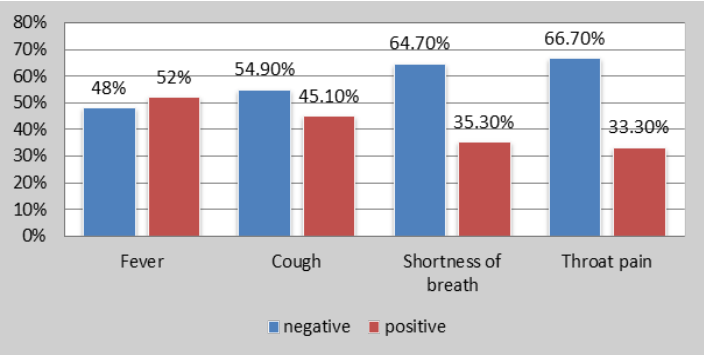

Figure 1: Distribution of Symptoms among RT-PCR positive group.

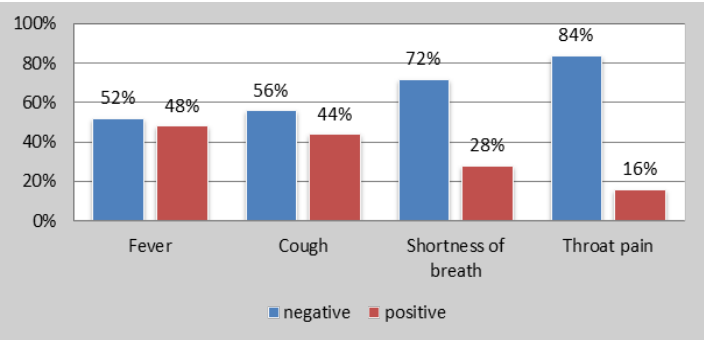

Figure 2: Distribution of Symptoms among the RT-PCR negative group.

Table 2: The sensitivity and specificity of ICT Ag compared with the RT-PCR technique.

ICT Ag results	Case	Control	Total	Sensitivity	Specificity	PPV	NPV
Positive	61	13	74	59.8%	48%	82.4%	22.6%
Negative	41	12	53				
Total	102	25	127				

PPV= Positive predictive value. NPV = Negative predictive value.

Table 3: The sensitivity and specificity of ICT Ab compared with the RT-PCR technique.

ICT Ab results	Case	Control	Total	Sensitivity	Specificity	PPV	NPV
Positive	33	13	46	32.4%	48%	71.7%	14.8%
Negative	69	12	81				
Total	102	25	127				

PPV= Positive predictive value. NPV = Negative predictive value

Association of ICT/Ag with symptoms among the RT-PCR positive group

Throat pain revealed a significant association with ICT /Ag (*P*-value 0.004) among the RT-PCR positive group, while no significant difference was detected for fever (*P*-value 0.352), cough (*P*-value 0.312), and shortness of breath (*P*-value 0.296) (Table 4).

Association of ICT/Abs with Symptoms Among the RT-PCR positive group

No significant association was detected for ICT/Abs with observed symptoms (*P*- value > 0.05) (Table 5).

Table 4: Frequency and significance of symptoms among the RT-PCR positive group detected by ICT/Ags.

Symptoms			Ag		Total	P. value
			Positive	Negative		
Fever	Positive	Count	34	19	53	0.352
		%	33.3%	18.6%	52.0%	
	Negative	Count	27	22	49	
		%	26.5%	21.6%	48.0%	
Cough	Positive	Count	30	16	46	0.312
		%	29.4%	15.7%	45.1%	
	Negative	Count	31	25	56	
		%	30.4%	24.5%	54.9%	
Shortness of Breath	Positive	Count	24	12	36	0.296
		%	23.5%	11.8%	35.3%	
	Negative	Count	37	29	66	
		%	36.3%	28.4%	64.7%	
Throat Pain	Positive	Count	27	7	34	0.004*
		%	26.5%	6.9%	33.3%	
	Negative	Count	34	34	68	
		%	33.3%	33.3%	66.7%	

Table 5: Frequency and significance of symptoms among the RT-PCR positive group detected by ICT/Abs.

Symptoms			Ab		Total	P. value
			Positive	Negative		
Fever	Positive	Count	19	34	53	0.432
		%	18.6%	33.3%	52.0%	
	Negative	Count	14	35	49	
		%	13.7%	34.3%	48.0%	
Cough	Positive	Count	19	27	46	0.080
		%	18.6%	26.5%	45.1%	
	Negative	Count	14	42	56	
		%	13.7%	41.2%	54.9%	
Shortness of Breath	Positive	Count	15	21	36	0.138
		%	14.7%	20.6%	35.3%	
	Negative	Count	18	48	66	
		%	17.6%	47.1%	64.7%	
Throat Pain	Positive	Count	15	19	34	0.073
		%	14.7%	18.6%	33.3%	
	Negative	Count	18	50	68	
		%	17.6%	49.0%	66.7%	

Discussion

When we compared the performance of the rapid serological test (Ag and Ab) to that of RT-PCR for the detection of SARS-CoV-2 infection, our findings showed that 59.8% (61/102) of the subjects tested positive based on the Ag test, whereas 32.4% (33/102) of the subjects tested positive based on serological (Anti COVID-19 IgG/IgM) test results, leading to a sensitivity of 59.8% for Ag and a 32.4% of for the serological test. For this reason, we compared its performance to standard RT-PCR testing and analyzed performance concerning the time of COVID-19-related symptoms. The design of our study allowed us to precisely analyze two aspects of the rapid serological test results with those of standard molecular testing and the relationship between (Ag), (IgG/IgM) expression, and the onset of clinical symptoms. The results reported showed that the precision of the Ag rapid test can detect the majority

of infectious cases, whether symptomatic or not, and that it is sufficiently rapid to maximize the effectiveness of case isolation and contact tracing. The results of Abs (IgM/IgG) were found to be unsatisfactory. Notably, only 32.2% (33/102) of the patients who tested positive for COVID-19 based on the molecular test results also tested positive for the serological test (Anti-COVID-19 Abs). This percentage is impressively similar. Negative serological test results in patients with a positive molecular test could mean that the patients are infected but have not yet reached the stage of immunoglobulin reaction development. In the current study, which aimed to measure the sensitivity and specificity of ICT/ IgG and IgM showed a low sensitivity as 32.4%, while it showed moderate specificity of 48% for both together. The negative predictive value for ICT/Ab was 14.8%, but the positive predictive value was 71.7%, thus indicates that the test (ICT) is moderately specific but low sensitive. Li et al., reported the sensitivity and specificity of IgM/IgG as 88.7% and 90.6%, respectively. They explained that the false-negative was due to low antibody concentration, the differences in immune response between individuals, the decrease and disappearance of IgM antibody after two weeks, and difficulties in knowing the exact time of infection. [19]. His study was in line with our findings, considering the variation in Sample size. Likewise, Xiang et al., reported a sensitivity and specificity of IgM/IgG as 82.4% and 100%, respectively [20].

On the other hand, Dohla et al., found that the sensitivity and specificity of IgM/IgG were 18.4% and 36.4%, and 91.7% and 88.9%, respectively, and additionally due to poor sensitivity, they concluded that COVID19 IgM/IgG rapid diagnostic test is not recommended for a patient with suspected COVID-19 [21]. Perhaps, the low sensitivity may be due to samples taken in the early onset of the disease. No significant association was determined for clinical symptoms among RT-PCR positive COVID patients tested by ICT/Ag, except for that of throat pain (*P*-value 0.004), which may reflect the high significance and importance of this feature for clinical diagnosis. On the other hand, for COVID patients tested by ICT/Ab, no significant difference was detected for symptoms in association with antibodies presence.

Limitation

The main limitation of the current study was the low number of sample size, there were differences in the type of SARS-CoV-2 antigen targeted in the assay between the studies. The cross-reactivity between SARS-CoV-2 and another human coronavirus might have happened.

Conclusion

The conclusion of the present study, depending on the laboratory resources, using antigen and antibody testing as a screening test for SARS-CoV-2 infection, is that the study revealed moderate sensitivity and low specificity for ICT/Ag and decreased values of both sensitivity and specificity for ICT/Ab compared with RT-PCR. A significant association was determined for throat pain with ICT-Ag within the COVID-19 positive group.

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