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Research Article

Validity of COVID-19 Rapid Tests in Iraq

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ABSTRACT

Background: Since the inception of the Coronavirus Disease-2019 (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) several methods were developed to identify and diagnose the disease such as rapid Polymerase Chain Reaction (PCR) test, rapid antibody test, and rapid antigen test. In Iraq, no study had evaluated the accuracy of the rapid antibody test before introducing it into the field.

Objective: To assess the validity of the rapid test (Dutch-made Biozek) used in Iraq through calculating the sensitivity, specificity, positive and negative predictive values on a sample of suspected COVID-19 cases.

Materials and Methods: A descriptive cross-sectional study conducted at two Primary Healthcare Centers (PHCs); 1000 individuals were included either because of the classical symptom of the disease and attended the PHC for testing or they were healthy individuals with close contact with a confirmed case at home or work. A structured questionnaire was filled in through direct interviews with the participants. Both rapid antibody test and PCR test were done simultaneously. The sensitivity, specificity, positive and negative predictive values were calculated.

Results: Around 85% aged 20-60 years with almost equal gender distribution. About 78% were symptomatic, and 32.6% had history of contact with a confirmed case. The sensitivity was 14% for Immunoglobin (Ig) G, 3.2% for IgM and 5.7% for both, compared to specificity which was 74.5% for IgG, 95.1% for IgM and 91.3% for both. While the Positive Predictive Value (PPV) was 32.8% for IgG, 36.6% for IgM, and 37% for both, and the Negative Predictive Value (NPV) was 49.3% for IgG, 52.5% for IgM and 52.1% for both.

Conclusion: Rapid antibody test used in this study which is not generally recommended for the early diagnosis of COVID-19 patients or in the screening programs. However, it can be used for epidemiological surveys purposes.

Keywords: COVID-19, Rapid antibody test, PCR test, Immunoglobin (IgG)

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INTRODUCTION

COVID-19 pandemic caused by SARS-CoV-2 was 1st reported in Chinese city of Wuhan and it later spread to affect almost every country in the world (Sohrabi C, *et al.*, 2020). World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) on March 11, 2020 (Sohrabi C, *et al.*, 2020) and by 14 February, 2021, the total infected people have crossed 108 million with more than 2.386 million deaths (WHO, 2020).

Since January 7, 2020 the standard for diagnosing COVID-19 as per WHO guidelines was the Nucleic Acid Amplification Test (NAAT) in Real Time Reverse Transcription- Polymerase Chain Reaction (RT-PCR) test using respiratory samples (Long C, *et al.*, 2020; Corman VM, *et al.*, 2020). PCR can detect the Ribonucleic Acid (RNA) of the virus 3-4 days after the appearance of symptoms and have a turn-around time of 24-48 hours (Long C, *et al.*, 2020). The average cost of performing PCR test ranges between $35.91 and $51.31 which vary from country-to-country (Weissleder R, *et al.*, 2020).

In the past, during several outbreaks with viral diseases such as SARS and Middle East Respiratory Syndrome (MERS), antibody-based tests have provided substitute and effective method of ultra-rapid detection (Iravani S, 2020). Similarly, earlier during the COVID-19 pandemic Aziz AB, *et al.*, 2020, several rapid diagnostic methods were developed to diagnose COVID-19 and identify its spread across the globe, such as rapid PCR test, rapid antibody test and rapid antigen test (Weissleder R, *et al.*, 2020; Peeling RW, *et al.*, 2020). Further, the price of rapid antibody and antigen tests is lower, compared with the price of the PCR test. This may explain the wide use of the rapid test in various countries around the world (Aziz AB, *et al.*, 2020).

On March 1, 2020 many developed and developing countries started using rapid antibody tests in sero-surveys to detect the possible spread of COVID-19 (Peeling RW, *et al.*, 2020). Following the appearance of symptoms, IgM antibodies can be detected in the patient's blood within 5-10 days while IgG antibodies can be detected after 12-14 days. The immunity response takes from 35-40 days to reach maturation stage which varies depending on the patient's immune status, presence of other diseases and other factors (Peeling RW, *et al.*, 2020; Azkur AK, *et al.*, 2020).

In Iraq, the 1st reported case of COVID-19 was reported on February 24, 2020 and by February 14, 2021 the total number of cases were found to be 6,41,628 while the total number of deaths were 13,164 (WHO, 2020; Andrey DO, *et al.*, 2020). Rapid antibody test for COVID-19 was introduced in Iraq on May 1, 2020 during active surveillance activities (Iraqi Centers for Disease Control and Prevention (CDC)). Rapid antibody test was introduced without assessment of its sensitivity, specificity, PPV and NPV. Those who were tested as positive for rapid antibody test were tested for COVID-19 using PCR to confirm the diagnosis. In contrast, those who were tested as negative were considered negative and no further testing was carried.

The current study aims to calculate the sensitivity, specificity, positive and negative predictive values of the rapid test used in Iraq and to determine if having COVID-19 symptoms or a history of contact with a confirmed case would affect these validity indicators.

MATERIALS AND METHODS

Study design

The study was conducted at two PHCs, Baghdad-Aljadidea and Al-Mustansiriya PHCs. Both the centers are located on the Eastern side of the capital Baghdad, under the health authority of Al-Rusaffa Directorate of Health (DOH). The catchment population of each center is around 70,000.

Study population

A consecutive sample of 1000 individuals who attended the two assigned PHCs were included. Most of the attendees were either suspected COVID-19 cases because of the classical symptom of the disease and had attended the PHC for testing or they were healthy individuals with close contact with a confirmed case at home or work.

Screening methods

Screening methods started with filling a questionnaire developed for this purpose and through the direct interview with the participants. The questionnaire included the following variables, basic demographic information, contact information, main clinical manifestations for the participants who were suspected COVID-19 cases and history of contact with confirmed COVID-19 case (home, work or other). Then the rapid antibody test was done by trained laboratory personnel. Result of the rapid test was classified as negative to both, IgM positive, IgG positive or positive to both. After that, a nasopharyngeal swab was obtained for PCR testing. On the 2nd day, we received and reported the PCR result which was classified as positive or negative. For equivocal results, we called the participant to attend and have a 2nd nasopharyngeal swab for the PCR test.

The commercial brand of the rapid antibody test used in this study was the Dutch-made biozek which was procured by the Iraqi Ministry of Health. Rapid antibody test was done following the instructions included with the rapid test cassette (Andrey DO, *et al.*, 2020; Celentano DD, 2023). PCR testing on the nasopharyngeal specimen was performed at the Central Public Health Laboratory (CPHL) where the Chinese-made Jinan-Babio PCR kit was used. Results of the rapid test were obtained on the spot and the participants were immediately informed while the results of the PCR test were obtained on the 2nd day and were communicated by telephone to the participants. There was 5.6% of the PCR test which was repeated and this is due to inadequate nasopharyngeal samples or inaccurate transporting of the samples to CPHL.

Statistical analysis

The information in the data collection tool was transferred to the Epi Info software for all the 1000 individuals composing the study sample. Data was analyzed later using both Epi Info and Excel spreadsheet. The following validity measures were calculated (Celentano DD, *et al.*, 2023).

Sensitivity=TP/TP+FN (TP=True Positive, FN=False Negative)

Specificity=TN/TN+FP (TN=True Negative, FP=False Positive)

PPV=TP/TP+FP

NPV=TN/TN+FN

RESULTS

The total sample size was 1000 individuals. Around 85% (848) aged between 20-60 years with almost equal gender distribution (males=504, 50.4%) were selected. Around 1/3rd of the study group was currently employed (n=385, 38.5%) and 96.3% were residing in Al-Rusaffa (Eastern side of Baghdad).

Most common symptoms were fever and cough (48.8%), sore throat (20.7%) and other symptoms were also seen in 30.5% of the participants. There were 224 (22.4%) participants with close contact with household member, while 102 (10.2%) were in close contact to co-workers (*Table 1*). Almost half of the participants (n=471, 47.1%) were positive for PCR.

The rapid test results found were, 68.5% negative and the remaining 31.5% were classified as 20.1% positive for IgG, 4.1% for IgM and 7.3% positive for both.

The overall sensitivity included 14% for IgG, 3.2% for IgM and 5.7% for both. The highest sensitivity was seen in individuals with other signs and symptoms which was 34.5% for both IgM and IgG and 32.8% for IgG (*Table 2*).

Table 1: Characteristics of the study population

Total	n=1000	Percentage (%)
Age		
0<20	72	7.20%
20<40	466	46.60%
40<60	382	38.20%
>60	80	8%
Gender		
Male	504	50.40%
Female	496	49.60%
Occupation		
Employee	385	38.50%
Housewife	264	26.40%
Student	114	11.40%
Free lancer	209	20.90%
Others	28	2.80%
Rapid test		
IgG positive alone	201	20.10%
IgM positive alone	41	4.10%

Both IgM and IgG positive	73	7.30%
Rapid test negative	685	68.50%
PCR test		
PCR positive	471	47.10%
PCR negative	529	52.90%
History of contact with a confirmed case		
With-household cases	224	22.40%
With-work cases	102	10.20%
No contact	674	67.40%
Symptoms		
Having symptoms	779	77.90%
Fever	426	25.90%
Cough	374	22.80%
Shortness of breath	100	6.10%
Loss of smell	92	5.60%
Loss of taste	67	4.10%
Headache	73	4.50%
Diarrhea	26	1.60%
Sore throat	339	20.70%
Fatigue	85	5.20%
Myalgia	45	2.80%
Vomiting	5	0.30%
Abdominal pain	7	0.40%
Asymptomatic	221	22.10%

Table 2: Sensitivity, specificity, NPV and PPV of a sample according to its category

Samples				**Total (1000)**	**Sensitivity (%)**	**Specificity (%)**	**NPV (%)**	**PPV (%)**
Rapid test		PCR						
IgG	Result	Positive	Negative					
	Positive	66	135	201	14%	74.50%	49.30%	32.80%
	Negative	405	394	799				
IgM	Positive	15	26	41	3.20%	95.10%	52.50%	36.60%
	Negative	456	503	959				
IgG and IgM	Positive	27	46	73	5.70%	91.30%	52.10%	37%
	Negative	444	483	927				
Asymptomatic								
Rapid test		PCR		Total (221)				
IgG	Result	Positive	Negative	25	4.60%	84.30%	57.70%	16%
	Positive	4	21					
	Negative	83	113	196				

IgM	Positive	0	3	3	0%	97.80%	60.10%	0%
	Negative	87	131	218				
IgG and IgM	Positive	1	1	2	1.10%	99.30%	60.70%	50%
	Negative	86	133	219				
Symptomatic								
Rapid test		PCR		Total (546)				
IgG	Result	Positive	Negative	168	26.10%	64.70%	47.60%	41.70%
	Positive	70	98					
	Negative	198	180	378				
IgM	Positive	41	48	89	15.30%	82.70%	50.30%	46.10%
	Negative	227	230	457				
IgG and IgM	Positive	50	65	115	18.70%	76.60%	49.40%	43.50%
	Negative	218	213	431				
Fever, cough and sore throat								
Rapid test		PCR		Total (126)				
IgG	Result	Positive	Negative		28.30%	63%	54.80%	35.70%
	Positive	15	27	42				
	Negative	38	46	84				
IgM	Positive	4	4	8	7.50%	94.50%	58.50%	50%
	Negative	49	69	118				
IgG and IgM	Positive	16	26	42	30.20%	64.40%	56%	38.10%
	Negative	37	47	84				
Other signs and symptoms								
Rapid test		PCR		Total (136)				
IgG	Result	Positive	Negative	47	32.80%	64.10%	56.20%	40.40%
	Positive	19	28					
	Negative	39	50	89				
IgM	Positive	11	14	25	19%	82.10%	57.70%	44%
	Negative	47	64	111				
IgG and IgM	Positive	20	26	46	34.50%	66.70%	57.80%	43.50%
	Negative	38	52	90				
Contact cases								
Rapid test		PCR		Total (326)				
IgG	Result	Positive	Negative	53	15%	82.70%	52.40%	43.40%
	Positive	23	30					
	Negative	130	143	273				
IgM	Positive	1	7	8	0.70%	96%	52.20%	12.50%
	Negative	152	166	318				
IgG and IgM	Positive	5	5	10	3.30%	97.10%	53.20%	50%
	Negative	148	168	316				

The overall specificity was found to be 74.5% for IgG, 95.1% for IgM and 91.3% for both. The highest specificity was 84.3%, 97.8%, 99.3% for IgG, IgM, both IgG and IgM respectively in asymptomatic individuals. PPV was 32.8% for IgG, 36.6% for IgM and 37% for both. Highest PPV was 50% for IgM in patients who reported fever, cough and sore throat and in both IgM and IgG in asymptomatic individuals as well as those with close contact to confirmed cases. The overall NPV was 49.3% for IgG, 52.5% for IgM and 52.1% for both. Highest value was 60.7% for both IgM and IgG in asymptomatic participants and 58.5% for IgM in patients who reported fever, cough and sore throat.

DISCUSSION

The study demonstrated low sensitivity of rapid test (14% for IgG, 3.2% for IgM and 5.7% IgM and IgG). Based on these values, there will be about 86%-96% of the patients who will be falsely assured that they have no disease which increases the risk of spreading the disease and leads to ineffective treatment of the cases.

In contrast, the specificity was moderate/high (74.5% for IgG, 95.1% for IgM and 91.3% for both IgM and IgG). These values indicated that there will be about 5%-25% of the non-infected individuals who might be falsely diagnosed as having the disease and risk the disease stigma and inappropriate case management.

Besides, the study shows low PPV of the rapid test (32.8% for IgG, 36.6% for IgM and 37% for both IgM and IgG). These values indicated that the certainty of positive test result was 63%-67%. In contrast, NPV was moderate (49.3% for IgG, 52.5% for IgM and 52.1% for both IgM and IgG), which indicates that the certainty of negative result of the rapid test was about 47%-50%.

In summary, the study finds that the specificity and NPV for rapid test is higher than the sensitivity and PPV, that is, the negative rapid test result is more likely to be true than the positive test result. Nevertheless, the value of certainty of the negative result is moderate.

These findings indicate that the use of the rapid test for the initial screening of suspected COVID-19 cases may not be clinically beneficial. Low sensitivity and PPV of the rapid test could be due to several reasons. First, the quality of rapid test kit used and whether it is approved by the Food and Drug Administration (FDA) (Weissleder R, *et al.*, 2020; WHO, 2020). Second, the method of taking the blood samples to conduct the test and the experience of health personnel who conducted and interpreted the test can be another factor affecting the overall validity indicators (Jiang Y, *et al.*, 2020). Third, time of conducting the test during the course of disease and whether the patient is still in incubation period or in the early or late days after the appearance of the manifestations; this is one of the most important factors affecting the result of the rapid antibody test (Li Z, *et al.*, 2020). Also, concomitant viral infection may lead to higher FP as a result of cross-reaction leading to inaccurate PPV (Hoffman T, *et al.*, 2020; Cunha LL, *et al.*, 2020).

Additionally, PPV of rapid test varies by the antibody type and the subgroup of the study sample. Highest PPV values were 50% for IgM in participants who reported fever, cough and sore throat which was 50% for both IgM and IgG in asymptomatic participants and contact cases. This means the ability of the rapid test in detecting the affected cases is not high, indicating that 50% of the cases have not been diagnosed. This would lead to the continuation of the spread of the disease among the population if the rapid test was used in diagnosing COVID-19 (Ong JS, *et al.*, 2020; Kissler SM, *et al.*, 2020).

High frequency (47.1%) of positive PCR results for COVID-19 among the study participants was because of selecting the individuals who were referred to the PHCs either because they had symptoms, or they were close contacts with confirmed cases and not a sample from the general population. This indicates the necessity of the effective monitoring and early detection of cases in these groups (Velay A, *et al.*, 2020; Torres I, *et al.*, 2021). Further, this study was conducted when the cases of COVID-19 were at the peak of transmission and Iraq was reporting high daily numbers.

Regarding the NPV, the study shows the highest results were 60.7% for both IgM and IgG in asymptomatic participants and 58.5% for IgM in participants who reported fever and cough, and sore throat, and that indicates the probability of having the diseases is 39%-41% among those who were tested, which means there are missed treatment and isolation measures (Velay A, *et al.*, 2020). This also leads to a wider spread of cases among the community due to the asymptomatic cases who interact with people without knowing that they are infected or work in many institutions, whether governmental or private sector (Bisoffi Z, *et al.*, 2020).

Alongside our study, many studies have been conducted about this subject, some results were quite similar to the results of our study, but others were not. A meta-analysis was conducted to compare the diagnostic accuracy of Enzyme Linked Immunosorbent Assay (ELISA), Lateral Flow Immunosorbent Assay (LFIA) or Chemiluminescent Immunosorbent Assay (CLIA), IgM and IgG. In comparison to PCR results, sensitivity was 84.3%, 66.0% and 97.8% for ELISA, LFIA and CLIA respectively; these results are higher than our results. One of the reasons that may explain this difference is the quality of the commercial brand of the rapid test kit used and also the technique for taking the samples whether it is whole blood, plasma, or serum. Also, in this study, the tests were done after two weeks or more of the onset of symptoms while in our study, the tests were performed directly after the appearance of symptoms.

In contrast, the specificity results reported by this study, which were 96.6%-99.7%, are somewhat similar to the results of our study (Bastos ML, *et al.*, 2020). Another meta-analysis found the sensitivity in the 1st week from the onset of symptoms to be 13.4%-50.3% and changed to 69.9%-98.9% after 3 weeks from the onset of symptoms. This contrasts with our findings for sensitivity and gives an idea of how limited is the ability of the rapid tests to diagnose COVID-19 earlier (Castro R, *et al.*, 2020). Also, the specificity in this study was 90.63% which is consistent with our results (Castro R, *et al.*, 2020). This was also confirmed in another study that found the sensitivity of the test to be 69% and 93.1% for IgM and IgG respectively, which is indicative that rapid testing is useful to assess if there is previous exposure to the SARS-CoV-2 virus, due to high IgG antibodies titer (Pal M, *et al.*, 2020; Vidal-Anzardo M, *et al.*, 2020).

Research indicates that the results of the rapid antibody test are not reliable enough, especially with asymptomatic people (Torres I, *et al.*, 2021). There are a lot of FN results seen when using this test which could be due to the low antibody concentrations (Kissler SM, *et al.*, 2020), the difference in individual immune response antibody production, and the decrease or disappearance of antibodies after 2 weeks of infection (Peeling RW, *et al.*, 2020). In some cases, it is difficult to know exactly when and for how long the patient was infected (Curigliano G, *et al.*, 2020). As a result, when the patient is tested, the IgM level might be well below its peak and is not detectable by this test (Pal M, *et al.*, 2020; Curigliano G, *et al.*, 2020).

Our findings suggest that it is better not to use the rapid test in issuing health certificates to travelers through designated centers and hospitals, or in population screening programs in PHCs in diagnosing cases of SARS-CoV-2. Similarly, rapid tests should not be used in the pre-operative investigations that are conducted on patients. In contrast, rapid antibody tests can be used in community-based surveys to determine the prevalence of the disease in silent or low prevalence areas. Moreover, it can be used in community and healthcare workers surveys to determine the persistence of antibodies several months after infection (Bastos ML, *et al.*, 2020; Plans V, 2020). This study is the first study of its kind conducted to evaluate the validity of the used Dutch-made (Biozek) rapid test kits in Iraq.

CONCLUSION

In conclusion, the Dutch-made biozek rapid antibody test used in this study cannot be adopted as an effective tool to identify COVID-19 patients attending the health institutions, or for early detection and screening of potentially infected individuals. Also, it is not suitable as a point of care test like prior to performing surgical operations. In addition, it cannot be used in issuing health certificates for travelers. Its main use should be limited to conducting sero-surveys and during epidemiological surveillance to identify areas that are likely to be endemic areas.

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ETHICAL APPROVAL

Informed consent was obtained from the participants at the beginning of the interview and the information was kept anonymous and used exclusively for the sake of the study. The results of the rapid test and the PCR test were timely delivered to the participants. The ethical approval was obtained from the Ethical Committee at the Center of Human Resources Development and Training, Iraqi Ministry of Health.

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