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Evaluation of four commercial COVID-19 diagnostic RT-PCR kits used in Libya

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ABSTRACT

Background: Real-time reverse transcriptase chain reaction is considered the most sensitive and specific assay for COVID-19 detection. This study was planned to compare and evaluate four commercially diagnostic kits. Material and methods: 92 Nasopharyngeal swabs collected from different symptomatic patients were studied in this project. The total nucleic acid (RNA) was extracted, and RT-PCR was done using four commercial diagnostic kits from different manufacturers, DANN and BGI (China), Hibrigen, and Bio-speedy (Turkey). Result: Although all 92 clinical samples were subjected to the same four diagnostic kits, the results revealed variations in the performance. The total number of positive cases was (42/92) for Da An Gene, BGI (36/92), Bio-speedy (31/92), and Hibrirgen (14/92).

تقييم ومقارنة أربع محاليل تشخيصية مختلفة لتشخيص فيروس كورونا المستجد COVID-19 المستخدمة في ليبيا

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الكلمات المفتاحية:
تفاعل البلمرة المتسلسل
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الكوفيد -19

الملخص

يعد تفاعل البلمرة المتسلسل الأكثر حساسية والمتعارف عليه الان في اكتشاف فيروس الكورونا المستجد COVID-19. و يعد عذا الاختبار من اهم الاختبارات المستخدمة في الكشف عن التحورات الجينيه في الفيروسات . الهدف من هذه الدراسة هي مقارنة وتقييم أربع مجموعات تشخيصية مختلفة استخدمت في جائحة كورونا ومدي فاعبية كل اختبار في الكشف عن الفيروس. المواد والطرق: تمت دراسة 92 مسحة من البلعوم الأنفي لمرضى يعانون من أعراض مختلفة لاتهاب الجهاز التنفسي ومشتبه باصباتهم بالفيروس . في هذا المشروع. تم استخراج الحمض النووي الكلي (RNA) ، وتم إجراء (RT-PCR تفاعل البلمرة التسلسلي باستخدام أربع مشغلات تشخيصية لشركات مختلفة، DANN و BGI (الصين) ، Hibrigen ، و Bio-speedy (تركيا). النتيجة: على الرغم من أن جميع العينات السريرية الـ 92 خضعت لنفس مجموعات التشخيص الأربعة ، إلا أن النتائج كشفت عن اختلافات في الأداء وحساسية كل مشغل للكسف عن الفيروس. كان العدد الإجمالي

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للحالات الإيجابية (92/42) لكل من Da An Gene و (36/92) BGI و (31/92) Bio-speedy و (Hibrirgen (14/92

1.Introduction

COVID-19 is a disease caused by novel coronavirus 2 (SARS-CoV-2) that first emerged in Wuhan city, China on December 2019 (Zhou et al 2020). However, the novel coronavirus can cause mild to moderate respiratory diseases, but some people may exhibit severe respiratory illness, which can be fatal and needs urgent medical intervention (Huang et al. 2020 and Xu Z 2020). Therefore, early detection of the virus may improve the outcome of the disease and can decrease the mortality rate. During the outbreak, the rapid and reliable technique is the core of medical decision-making for asymptomatic and symptomatic people with COVID-19. Nowadays, the Real-time reverse transcription-polymerase chain reaction (RT-qPCR) is considered the golden standard technique used to detect this virus (Corman 2019). The ORF1ab/RdRp, E, N, and S genes are the target genes mainly used for COVID-19 detection by RT-PCR (Bahrami 2020 and van Kasteren 2020). Today, various commercial diagnostic kits COVID-19 are available for early diagnosis and treatment of the COVID-19 patients and to prevent further spread of the virus among the people (WHO 2021). During the last two years, several diagnostic kits have been tried to detect the presence of the virus and gave unsatisfactory results. However, the patient showed already signs of COVID-19 on computed tomography images (Tahamtan 2020, Reusken 2019). Therefore, to avoid reporting the false positive and false negative, which may lead to further spread of the virus in the community, a combination of sensitive and specific kits was necessary for accurate diagnoses. Recently, it has been noticed that this virus can quickly evolve to avoid a harsh environment (WHO 2020). The mutation in the virus genome, which was reported in December 2020, had led to the emergence of new variants, UK variant and other variants (African variant) (Galloway 2021, National Institute of Infectious Diseases (NIID) 2021 and WHO 2020). These variants have significant mutations, mainly the gene encoding the spike (S protein) (Li, Q 2020, Pillay 2020). In this study, we compared four different commercial kits used for COVID-19 detection, and we showed that the results could be different according to the applied kit. Based on our results, we also suggest that different kits should be tried to get accurate and reliable results for routine diagnosis.

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2. Material and methods

2.1 Sample Collection and study design

This study was designed and conducted at PCR unit, microbiology department, Sebha medical center, Sebha, Libya, during January-June 2021. Ninety-two (92) nasopharyngeal swabs from suspected COVID-19 patients were collected in 3ml viral transport media for confirmation by RT-QPCR test at the COVID-19 laboratory unit.

2.2 Nucleic acid extraction and RT-PCR test

Nasopharyngeal swabs were collected from all suspected cases admitted to the Triage department between September and December 2020and confirmed to have COVID-19 by RT-PCR test at PCR unit in the Laboratory at Sebha Medical Center. According to the manufacturer's instructions, the viral RNA was extracted from the 120 - 200 µL of the samples using Da An Gene (Co., Ltd. of Sun Yat-sen Universit) extraction kit and NuActor machine (Boditech med. Inc. Chuncheon-Si, Gang-won-do 24398, Korea. The RT-PCR amplification was performed using four commercial RT-PCR diagnostic kits. Notably, all of the PCR kits that we had selected for our analysis were provided by the Biotechnology research center, Tripoli, Libya (BTRC), including DANN (Detection kit for 2019Noval Coronavirus*2019-nCov) RNA (PCR –Fluorescence Probing), Real-time fluorescent RT-PCR kit for 2019 nCOV (BGI), Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit Bioeksen, and Hibrirgen. All PCR assays were done according to the instructions provided by the manufacturers. All PCR reactions were performed using the same real-time PCR machine, Rotor-Gene Q (Qiagen) machines to ensure quality control. In addition, for quality assurance, negative, and positive controls provided by each manufacture were included in each assay. Each sample's cut-off threshold (Ct value) was recorded according to each applied kit. The results and features of all four commercially tested PCR kits are found in Tables 1 and 2.

2.3 Data analysis

For statistical analysis, collected data were analyzed using SPSS, version 24. Descriptive Analysis and Analysis of variance (ANOVA) test detected the significance between RT-PCR kits. Any *p*-values ≤0.05 were considered statistically significant.

2.4 Analysis of the Results

Nasopharyngeal swabs from 92 highly suspected patients with Covid-19 were collected and transported to the laboratory for detection by RT-PCR technique. All samples were tested for Covid-19 using four commercial RT-PCR diagnostic kits (**Table 1**). To analyze the results, we followed the instructions provided by each kit according to the manufacturers supplied. For Da An Gene (Detection kit for 2019Noval Coronavirus*2019-nCov) RNA (PCR – Fluorescence Probing) Da An Gene diagnostic kit, the sample was considered positive for Covid-19 if the Ct value in the FAM (ORF1ab gene) and VIC (*N gene*) channels is not more than 40 and there was clear amplification curve. For Real-time fluorescent RT-PCR kit for 2019nCOV (BGI) diagnostic kit, the specimen was recorded as positive for Covid-19 if Ct value of the test sample in the FAM channel (*ORF1ab* gene) is not higher than 38. Regarding Hibrigen covid-19 RT-PCR detection kit, the sample was considered as positive fro Covid-19 if the Ct value in FAM channel (*RdRp gene*) and Hex channel (N gene) is not more than 40 with clear amplification curve. Using Biospeedy diagnostic kit, obvious amplification curve in the HEX channel (*ORF1ab* gene), with a Ct value < 38.0 was diagnosed as positive Covid-19.

3. Results

In this study, according to a single and multiple gene positivity, our analysis was done and details are found in Table 2. We observed that all sample showed almost the same Ct values although different assays were used. Regarding Da An Gene (Detection kit for 2019Noval Coronavirus*2019-nCov) RNA (PCR –Fluorescence Probing) ORF1ab and N genes were detected in 46% (42/92) of all cases. Out of 42 positive samples, N gene was detected in 40 samples, while ORF1ab gene was detected in 38 cases. Since BGI (Real-time fluorescent RT-PCR kit for 2019 nCOV) diagnostic kit is based on derection of one gene, ORF1ab, this gene was detected in 39% (36/92). By using Biospeedy kit, RdRP gene was dtected in only 34% (31/92). Although the the primers in Hibrigen diagnostic kit were designed to find two genes, our data showed that N and RdRp genes could only be detected in 15% (14/92). In Hibrigen assay, N gene was detected in 12 samples out of 14 and RdRp gene has also been detected in 12 samples. Further, the Positive results according to only ORF1ab gene was higher for the Real-time fluorescent RT-PCR kit for 2019 nCOV (BGI) assay in comparison to Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit Bioeksen. On the other hand, there was significant difference observed between positive results of Da An Gene and Hibrigen assays according to multiple genes. However, The difference between used kits was a significant and the P-value was < 0.05.

4. Discussion

The outbreak of COVID-19 has become a global public health problem, and early detection of the virus has been considered a crucial step for controlling its spread (Wang 2020). Although the

sampling and transportation of the viral specimen are considered important for the detection of the virus, yet the quality of the kits, have nowadays showed their important role in the diagnosis (Tahamtan 2020). The main problem with the real-time RT-PCR test is the risk of false-negative results, and many cases were typically presented with Covid-19 symptoms and signs, and identical specific computed tomography (CT) images could not be confirmed RT-PCR (Wang 2020). False-negative results are essential for isolation and, subsequently the management of COVID-19 patients. Moreover, the false-negative results may increase the risk of transmission and spreading the virus. Therefore, the negative result obtained by RT-PCR test cannot exclude the possibility of Covid-19 infection, and a retest of the suspected patient should be considered (Arevalo-Rodriguez 2020, Mouliou 2021).

In this study, we compared four different available commercial RT-PCR kits using a patient sample were admitted to the triage department, Sebha medical center. Comparing the performance of used diagnostic kits in this study, we observed the highest diagnostic accuracy with Da An Gene COVID-19 RT-PCR kit, compared with other assays included in this study.

Based on the difference in the number of positive samples, there were 28 more with Da An Gene testing than Hibrigen, although both detect N genes. Further analysis showed that Ct values that refer to amplification of the N gene were closely similar in both Da An Gene and Hibrigen kits. Using Da An Gene assay, the N gene was found in 40 samples out of 42 positive cases, while the ORF1ab gene was detected in 38 samples. This result is similar to what has been obtained by Wang 2021 and Lucila 2021, where they detected similar analytical sensitivities of the Da An Gene assay compared to other kits used in their study. However, this sensitivity to N gene is probably due to the abundance of sub-genomic N gene messenger RNAs compared to other targets (Ogando 2020 and Ong DSY2020). Moreover, our results revealed that the Ct values were similar in the ORF1ab gene in the Real-time fluorescent RT-PCR kit for 2019nCOV (BGI) and Da An Gene assays, although the positive result was with Da An Gene was more than BGI. Further, the positive results of the Bio-speedy SARS –COV-2 qPCR kit compared to Hibrigen regarding the RdRp gene was higher.

It is well known that the Ct values are referred to the concentration of virus in the body, and Ct value is inversely correlated to the viral load (Yu F 2020). Furthermore, a low Ct value has been recorded with high disease severity and infectivity (Magleby 2020). It has also been reported that Ct above 34 are often found in those patients who are no longer infectious and do not excrete infectious viral particles anymore (La Scola 2020). In this study, most of the samples showed Ct value less than 34, and only 9 were above 34 cycles with N gene. This result agrees with other studies that reported the correlation between the severity and the viral load (Zheng 2020, Liu 2020). Since all samples in this study were collected from hospitalized patients complaining of respiratory symptoms, we found a significant correlation between the viral load and the severity of the disease.

On the other hand, many other studies have reported the opposite and they found no relationship between Ct values and disease severity (Zou 2020). In Conclusion, because of the laboratory's crucial role in the surveillance and assessment of the outbreak during the pandemic, it is crucial to maintain regular monitoring of the diagnostic kits used in the routine investigation of the COVID-19. However, the manufacturers need to improve their products further to increase the diagnostic capability of low viral load and avoid false-negative results.

5.Conclusion:

the false-negative result for some applied kits was high, reflecting the kits' efficiency. However, the manufacturers need to improve the product performance further to increase the diagnostic capability of low viral load and avoid false-negative results.

Conflicts of Interest:

The authors declare no conflict of interest.

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Figures and Tables

Table 1: RT-PCR program for different four diagnostic kits used in this study

Type of kit	Program				
	Hold 1 (1 cycle)	Hold2 (1 cycle)	Cycling		
				Denaturation	Anneal/ extend
Da An Gene (Detection kit for 2019Noval Coronavirus*2019-nCov) RNA (PCR –Flourescence Probing)	50°C for 15 min	95°C for 15 min	45 cycles	94°C for 15 sec	55°C for 45 sec
Real-time fluorescent RT-PCR kit for 2019 nCOV	50°C for 20 min	95°C for 10 min	40 cycles	95°C for 15 sec	60°C for 30 sec
Biospeedy	52°C for 5 min	95°C for 10 sec	40 cycles	95°C for 1 sec	55°C for 30 sec
Hibrigen	50°C for 15 min	95°C for 15 min	45 cycles	94°C for 15 sec	55°C for 45 sec

Table 2: Comparison of Specific standards of the four COVID-19 RT-PCR diagnostic kits

	Name of kit	Manufacture	Target genes	Kit interpretation	Percentage of Positive cases
I	Detection kit for 2019Noval Coronavirus*2019-nCov) RNA (PCR –Flourescence Probing) Da An Gene	China	N and ORF1ab	Ct < 40 Positive	46%
II	Real-time fluorescent RT-PCR kit for2019nCOV (BGI)	China (BGI Genomics)	ORF1ab	Ct < 38 Positive	39 %
III	Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit Bioeksen	Istanbol	RdRP	Ct < 38 Positive	34%
IV	Hibrigen	Kosuyolu Mih. Katip Salih Sk. No. 11Kadiköy- istanbul Istanbul	N and RdRp gene	Ct < 40 Positive	15 %

Abbreviations: *E* = envelope protein gene, *N* = nucleocapsid protein gene and ORF1ab = open reading frame 1ab, of the SARS-CoV-2 genome

Figures:

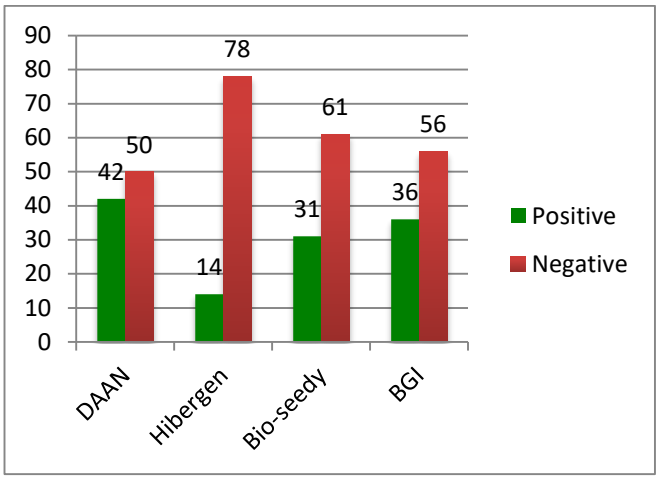

Figure 1: Comparison between results obtained from four different commercially available molecular kits for COVID-19 detection